Norrislide: Convergent Total Synthesis and Preliminary Biological Investigations

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NORRISOLIDE: CONVERGENT TOTAL SYNTHESIS AND PRELIMINARY BIOLOGICAL INVESTIGATIONS

a dissertation

by

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submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

August 2009

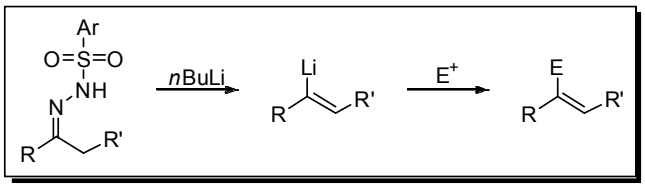
Norrisolide: Convergent Total Synthesis and Preliminary Biological Investigations

Krista Elizabeth Granger

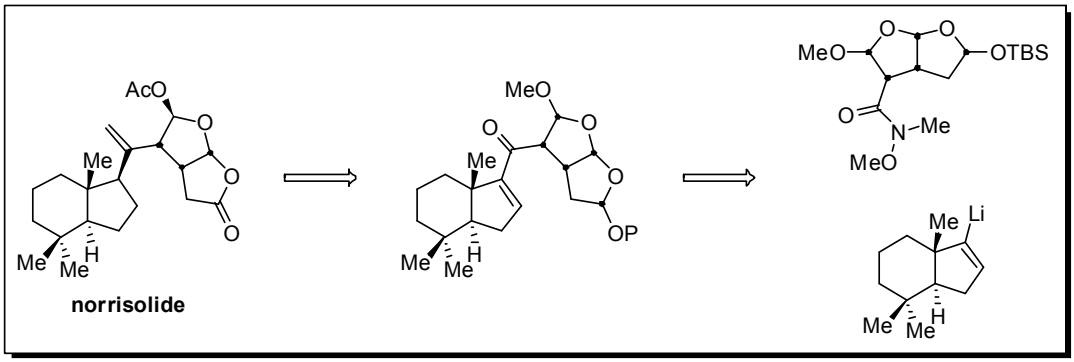
Thesis Advisor: Professor Marc L. Snapper

Abstract

- **Chapter 1:** A review of Shapiro reactions as a coupling strategy in natural product total synthesis. The syntheses of lycoramine, galanthamine, yuehchukene analogues, ovalicin, studies toward the ingenol core, haemanthidine, pretazettine, tazettine, crinamine, Taxol, colombiasin A, elisapterosin B, the AB ring fragment of spongistatin 1 and 8-epipuupewhedione are discussed.



- **Chapter 2:** The convergent total synthesis of the marine natural product norrisolide is described. Both subunits, the hydrindane core and the norrisane side chain, are prepared in an asymmetric fashion through kinetic resolution and enantioselective cyclopropanation, respectively. A Shapiro reaction couples the two fragments and a Peterson olefination installs the 1,1-disubstituted olefin.



- **Chapter 3:** Preliminary experiments to isolate the biological target of norrisolide through reductive alkylation and tritium labeling are investigated. Further experiments are proposed to shed light on the primary norrisolide-protein interactions.

Acknowledgements

I was first motivated to become a chemist in my freshman year of college by Prof. Jim Peploski. Dr. Jim always made chemistry seem so exciting and fun, and was always full of enthusiasm for teaching. The same enthusiasm was present when I began to work in the lab of Prof. Devon Shipp during my sophomore year, where I continued to learn and do chemistry until I graduated.

After seven years at Boston College, there are so many people I need to thank. I must begin with Prof. Marc Snapper for the opportunity to work on this total synthesis, along with the encouragement he gave during all of the rough points. Additionally, many thanks go to Dr. Rebecca Casaubon for starting the synthesis of norrisolide and welcoming me onto the project as a first year. Becky was a patient teacher in all things, and I appreciate her showing me the ropes in the lab.

I was fortunate to be part of a large incoming class of graduate students at BC. I can't imagine this experience without my classmates, especially those that joined the Snapper lab. Manami Shizuka, Ryan Murelli, Andrew Leyhane, and Yu Zhao were great classmates to share my time in the Snapper lab with, in addition to being in-house entertainment. I thank all of the others that I have spent time with in the Snapper lab. I always enjoyed coming to lab and having a variety of people to talk to about chemistry.

I thank Tim Gipson for his support these past two years, especially in these last few months. I'm not sure that I would have been able to make it through the last push towards writing and defending without having you to lean on. Thank you for listening to

my many frustrations about chemistry, even though most of the time I'm sure you had no clue what I was talking about.

I thank my family for being my support system these past few years. I wouldn't have been able to make it through graduate school without the encouragement and love. Mom, Dad, Tamara and Brett: There are not words to express how grateful I am for all that you have done for me. My extended family has also been a great source of support, including my grandparents, aunts, uncles and cousins.

List of Abbreviations

Å	angstrom
Ac	acetate
ATP	adenosine triphosphate
BHT	2,6-di-*tert*-butyl-4-methylphenol
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BINOL	1,1'-bi-2-naphthol
Bn	benzyl
Boc	*t*butyl carbamate
Bu	butyl
CBS	Corey-Bakshi-Shibata
Ci	curies
Cp	cyclopentadienyl
CSA	camphorsulfonic acid
Cy	cyclohexyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DDQ	dichlorodicyanoquinone
DIBAL	diisobutylaluminum hydride
DIEA	N,N-diisopropylamine
DMAP	4-dimethylaminopyridine

DMF	N,N-dimethylformamide
DMS	dimethylsulfide
DMSO	dimethylsulfoxide
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ER	endoplasmic reticulum
Et	ethyl
GDP	guanosine diphosphate
GTP	guanosine-5'-triphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMDS	hexamethyldisilazide
HMPA	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
IEC	ion-exchange chromatography
*i*Pr	isopropyl
IR	infrared
LAH	lithium aluminum hydride
LDA	lithium diisopropylamide
*m*CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MeOH	methanol

Mes	2,4,6-trimethylphenyl
MOM	methoxymethyl ether
Ms	methanesulfonyl
MS	molecular sieves
*n*Bu	butyl
NBS	*N*-bromosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMR	nucleur magnetic resonance
NRK	normal rat kidney
PAGE	polyacrylamide gel electrophoresis
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
PhCl	chlorobenzene
PhF	flurobenzene
PhH	benzene
PIPES	piperazine-1,4-bis(2-ethanesulfonic acid)
PMB	*para*-methoxybenzyl
PMSF	phenylmethanesulfonyl fluoride
PTSA	*para*-toluenesulfonic acid
pyr	pyridine
RCM	ring-closing metathesis

rt	room temperature
SAR	structure activity relationship
*s*Bu	*sec*-butyl
SEC	size exclusion chromatography
SDS	sodium dodecyl sulfate
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
*t*Bu	*tert*-butyl
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromotagraphy
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Tris	tri*iso*propyl sulfonyl
Ts	*para*-toluenesulfonyl
UV	ultraviolet

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Chapter 3: Cellular Interactions of Norrisolide: Preliminary Investigations

Chapter 1

The Shapiro Reaction as a Fragment Coupling Strategy in Convergent Natural Product Total Synthesis

1.1 Introduction

Total syntheses are often optimized by designing a convergent route. A convergent synthesis frequently improves the overall yield of the synthesis and conserves precious intermediates. Ideal couplings will happen late in the synthetic strategy using advanced intermediates with high yield and high selectivities. However, for large molecules, individual fragments may also be prepared in a convergent fashion, further improving the efficiency of the synthesis. Many methods exist for these fragment-coupling reactions, with C-C bond formation among the most challenging.

Syntheses commonly employ transition metal-mediated reactions for C-C bond formation that are both efficient and powerful. The Shapiro reaction shares a similar disconnection to these reactions. In many instances, the Shapiro reaction has advantages over transition metal-mediated reactions. Purification is simplified; no metal catalysts or ligands need to be removed at the end of the reaction. Less forcing conditions are required, as the Shapiro reaction is usually carried out at low temperatures (at or below 0 ºC), compared to the often elevated temperature of metal-mediated reactions. Finally, the Shapiro reaction is more environmentally friendly, not relying on stoichiometric heavy metals (such as tin in the Stille reaction) or other toxic additives such as arsenic.

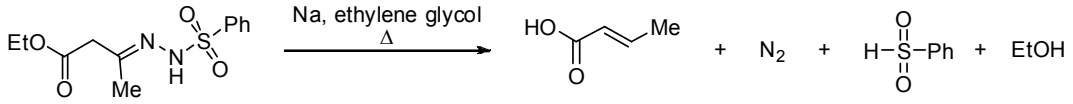
This review will examine convergent natural product total syntheses, including advanced studies toward natural products that employ the Shapiro reaction. To highlight the power of the strategy in convergent synthesis, only syntheses that bring together major fragments of the natural product targets have been included.

1.2 Background and History of the Shapiro Reaction

In 1952 Bamford and Stevens reported the decomposition of toluene sulfonyl hydrazones.[1] This initial reaction produced trace amounts of crotonic acid (**1.2**) from **1.1** through reaction with sodium in hot ethylene glycol (Scheme 1.1). Bamford and Stevens also reported that simpler ketones, such as acetone and cyclohexanone, could also be transformed to their phenyl sulfonyl hydrazones and treated under the same conditions to give propylene and cyclohexene, respectively.

Fifteen years later, Shapiro reported an improved procedure to provide the same transformation.[2] Treatment of camphor tosylhydrazone (**1.3**) with at least two equivalents of methyl or *n*-butyl lithium in hexanes provided 2-bornene (**1.8**) in quantitative yield (Scheme 1.2). This reaction was successful with a variety of simple

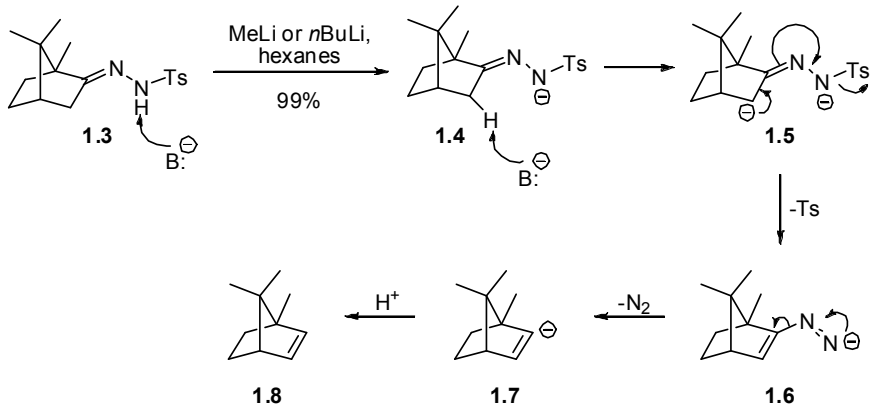
Scheme 1.1 Bamford and Stevens' reaction of an arylhydrazone to form crotonic acid.



1.1 **1.2**

[1] Bamford, W. R.; Stevens, T. S. *J. Chem. Soc.* **1952**, 4735-4740.
[2] Shapiro, R. H.; Heath, M. J. *J. Am. Chem. Soc.* **1967**, *89*, 5734-5735.

Scheme 1.2 Shapiro reaction of camphor tosylhydrazone with alkyl lithium reagents.



starting ketones, such as 2-methyl cyclohexanone and cholesterol derivatives. To begin the reaction the amine is deprotonated with the alkyl lithium base to give **1.4**, followed by formation of dianion **1.5**. Elimination of the sulfonyl group followed by loss of molecular nitrogen provides **1.7**, which upon proton quench gives olefin **1.8**.

Although yields to form proton-quenched olefins were high, attempts to trap the vinyl lithium intermediate with deuterium through a D_2O quench were met with low deuterium incorporation, except in the case of the most sterically hindered substrates. It was hypothesized that the vinyl carbanion could abstract a proton from ethereal solvents.[3] Changing the solvent to hexanes only slightly alleviated the problem, leading to the conclusion that the carbanion intermediate could also abstract an α-proton from another molecule of the starting tosylhydrazone. This evidence is consistent with the success of the sterically hindered substrates.

Without a general method to trap a variety of electrophiles for a wide range of substrates the synthetic utility of the Shapiro reaction was limited. Bond and coworkers

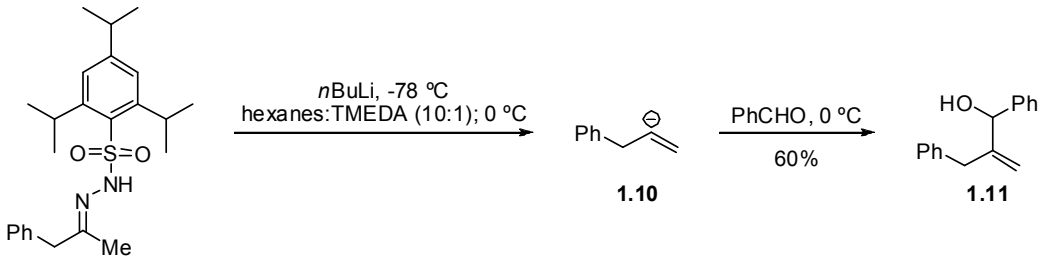
[3] Shapiro, R. H.; Heath, M. J. *J. Org. Chem.* **1974**, *39*, 2302-2303.

found two solutions to this problem. First, the use of tetramethylethylenediamine (TMEDA) as a cosolvent with hexanes allowed for high deuterium incorporation after quenching with D_2O.[4] Secondly, changing from a tosylhydrazone to a 2,4,6-triisopropylbenzene sulfonyl (trisyl) hydrazone greatly improved the reaction in many respects, due to increased steric bulk of the hydrazone.[5]

The additional bulk of the aromatic ring had many advantages. The isopropyl groups block deprotonation of the aromatic ring. In the past, excess base was added to the reaction to account for this side reaction; however the excess base would also alkylate the electrophile coupling partner. Therefore, excess electrophile was often added as well. The side products from these excess reagents could sometimes be problematic to separate from the desired product. Changing to the trisylhydrazone eliminated these challenges, making this reaction much more synthetically useful.

The trisylhydrazone diianion also decomposes much more readily to the vinyl lithium species. This decreases the amount of time that the vinyl anion and the hydrazone with its labile α-proton are both present, thus increasing the desired

Scheme 1.3 Shapiro reaction followed by benzaldehyde trap employing a trisylhydrazone.



1.9 **1.10** **1.11**

[4] Stemke, J. E.; Bond, F. T. *Tetrahedron Lett.* **1975**, *16*, 1815-1818.

incorporation of the electrophile. Indeed, Bond saw that the reaction of **1.9** with two equivalents of *n*-BuLi at –78 ºC followed by warming to 0 ºC and treatment with benzaldehyde gave **1.11** in 60% yield with less than 5% of the other regioisomer (Scheme 1.3).[6]

With these improvements, electrophiles can easily be coupled to the vinyl anion resulting from the treatment of trisylhydrazones with base, opening the door for a variety of coupling reactions in the realm of total synthesis.

1.3 Syntheses of (±)-Lycoramine and (±)-Galanthamine

The recent syntheses of (±)-lycoramine (**1.16**) (Scheme 1.4) and (±)-galanthamine (**1.23**) (Scheme 1.5) by the Tu group exemplify the use of the Shapiro reaction early in a synthetic effort.[7,8] Both alkaloids feature a unique tetracyclic framework with interesting biological properties; galanthamine has been approved for treatment for Alzheimer's disease.[9]

The synthesis of (±)-lycoramine (**1.16**) began with the tosylhydrazone **1.12**. Treatment of the tosylhydrazone with *n-butyl* lithium in TMEDA followed by addition of TBS protected *o*-vanillin gave the allylic alcohol **1.13** in 75%, installing two of the four

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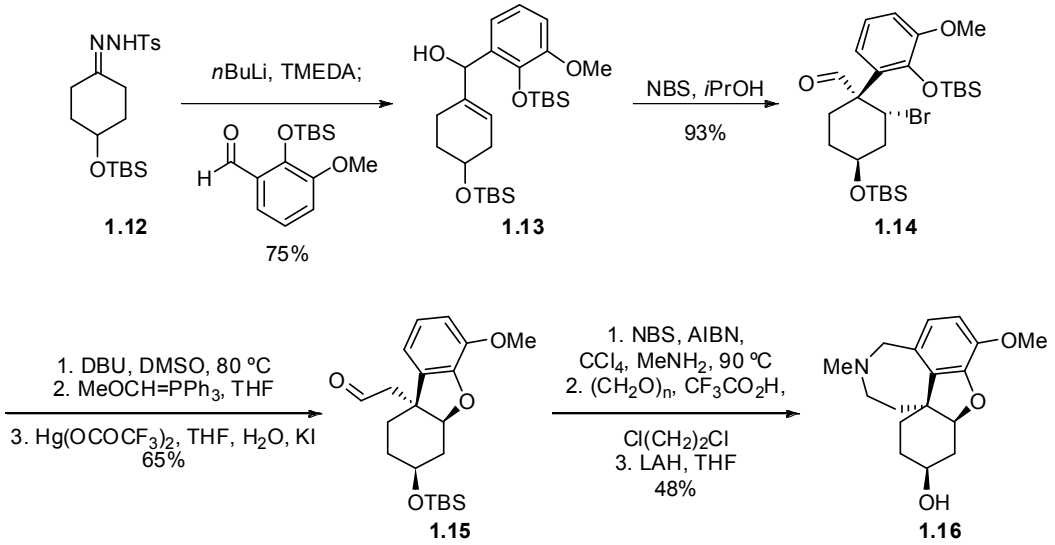
[5] Chamberlin, A. R.; Stemke, J. E.; Bond, F. T. *J. Org. Chem.* **1978**, *43*, 147-154.
[6] The regioselective deprotonation from the less hindered position is described in ref. 2. For another look at this reactivity, see: Corey, E. J.; Lee, J.; Roberts, B. E. *J. Am. Chem. Soc.* **1997**, *38*, 8915-8918.
[7] Hu, X.-D.; Tu, Y.-Q.; Zhang, E.; Gao, S.; Wang, S.; Wang, A.; Fan, C.-A.; Wang, M. *Org. Lett.* **2006**, *8*, 1823-1825.
[8] Fan, C.-A.; Tu, Y.-Q.; Song, Z.-L.; Zhang, E.; Shi, L.; Wang, M.; Wang, B.; Zhang, S.-Y. *Org. Lett.* **2004**, *5*, 4691-4694.
[9] Rainer, M.; *Drugs Today*, **1997**, *33*, 273-279.

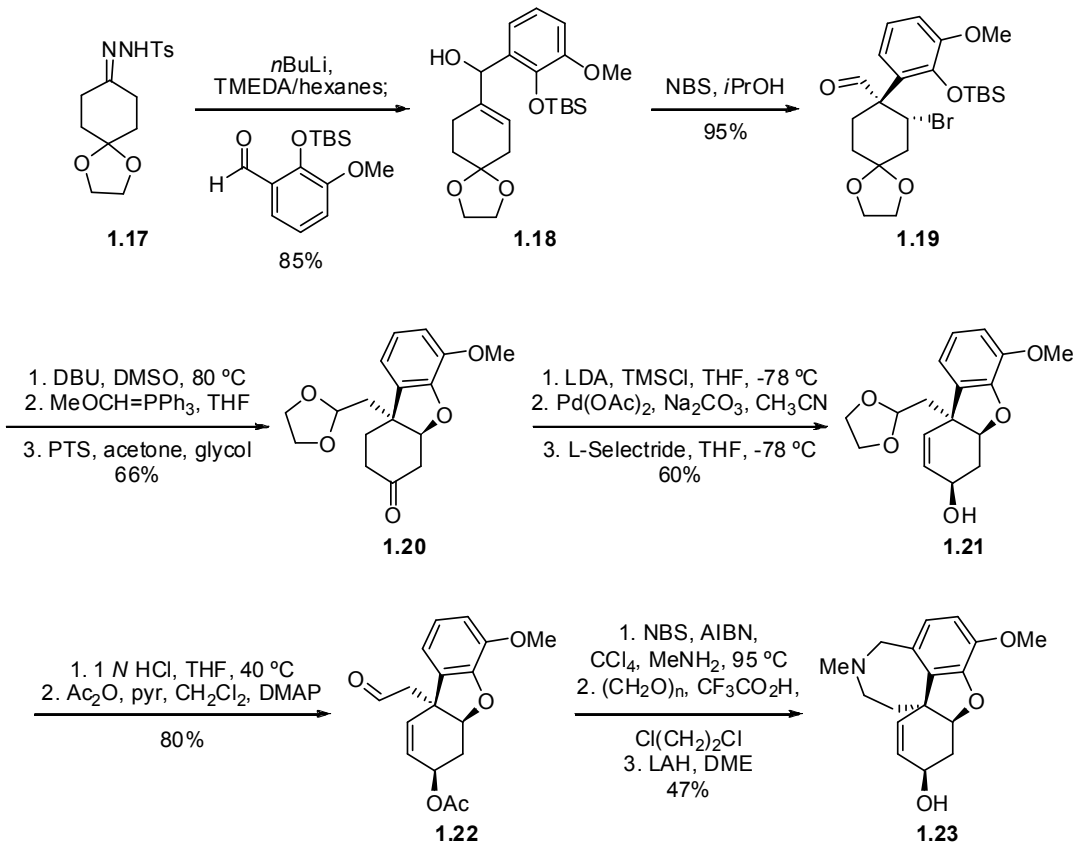
rings present in the natural product. Allylic alcohol **1.13** is also the key intermediate of the synthesis, undergoing a semipinacol rearrangement upon exposure to *N*-bromosuccinimide. After this key connection was made the five-membered ring was closed and the aldehyde homologated through a two-step procedure, giving **1.15**. Formation of the acyl bromide, treatment with dry methylamine and a modified Pictet-Spengler reaction with formaldehyde gave a lactam intermediate, which was reduced with lithium aluminum hydride to give the natural product **1.16**.

The total synthesis of (±)-galanthamine (**1.23**) follows a similar path to that of (±)-lycoramine, starting with a similar tosylhydrazone (**1.17**). A Shapiro reaction, again with TBS protected *o*-vanillin gave the key intermediate that underwent a semipinacol rearrangement to give **1.19**. A Wittig olefination followed by concomitant deprotection of the ketone and then protection of the aldehyde gives **1.20**, installing three of the four rings present in the natural product. Oxidation to the enone followed by 1,2-reduction

Scheme 1.4 Tu's synthesis of (±)-lycoramine starting with a Shapiro reaction



Scheme 1.5 Tu's synthesis of (±)-galanthamine starting with a Shapiro reaction



with L-Selectride gave allylic alcohol **1.21**, which underwent subsequent aldehyde deprotection and acylation, giving **1.22**. Installation of the seven-membered ring was done analogous to that of (±)-lycoramine, yielding **1.23**.

These two syntheses show the power of a Shapiro reaction utilized at the beginning of a total synthesis. Two vital segments of the natural products were brought together to give the allylic alcohol functionality desired for the key synthetic step.

1.4 Synthesis of (±)-Haemanthidine, (±)-Pretazettine, (±)-Tazettine and (±)-Crinamine from a common intermediate

Another example of an early stage Shapiro reaction from the Tu group that brings together the bulk of the carbon framework is in the syntheses of (±)-haemanthidine (**1.32**), (±)-pretazettine (**1.33**), (±)-tazettine (**1.34**) (Scheme 1.6) and (±)-crinamine B (**1.36**) (Scheme 1.7) by the Tu group.[10] The syntheses of all four natural products began with a Shapiro reaction between **1.24** and piperonal (**1.25**), giving secondary alcohol **1.26**, which contains most of the carbons present in the natural products. A semipinacol rearrangement promoted by NBS gave **1.27**, installing the difficult to access quaternary center.

Addition of cyanide to the aldehyde, reduction with lithium aluminum hydride and protection with acetone gave **1.28**. Elimination of the bromide with DBU was followed by deprotection of the N,O-acetal and the ketal under acidic conditions. The deprotection unmasks an enone which was then attacked by the free amine. Boc protection of the resulting secondary amine gave **1.29**.

Generation of the enone **1.30** took three steps. TMS protection of the free alcohol was followed by formation of the enol ether and finally reaction with palladium acetate installed the enone. Enone **1.30** underwent a 1,2-reduction with L-Selectride, and was protected as the methyl ether with inversion of stereochemistry. The TMS ether was then deprotected and replaced with an acetate group. Finally, Boc deprotection and formylation gave **1.31**.

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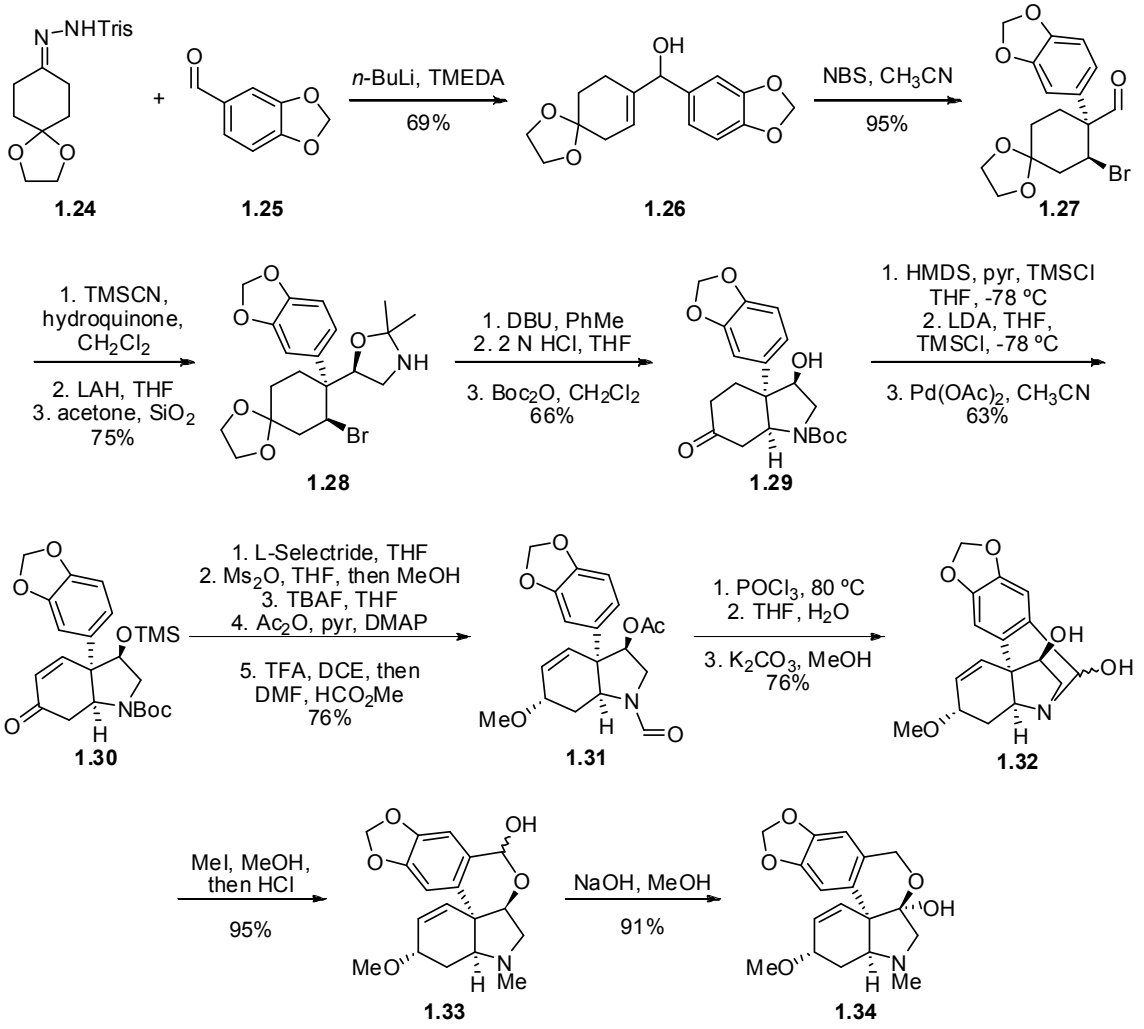
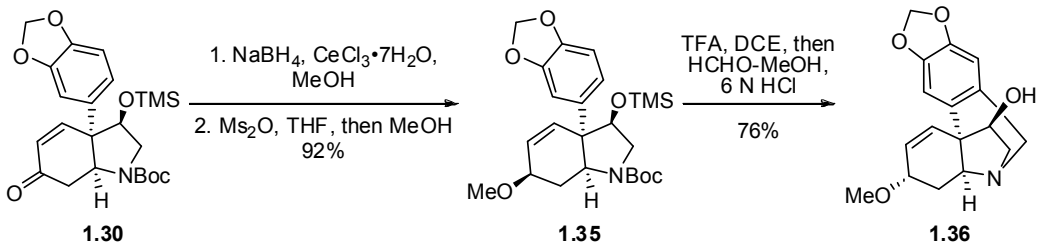
[10] Zhang, F.-M.; Tu, Y.-Q.; Liu, J.-D.; Fan, X.-H.; Shi, L.; Hu, X.-D.; Wang, S.-H.; Zhang, Y.-Q. *Tetrahedron*, **2006**, 9446-9445.

Scheme 1.6 Tu's syntheses of haemanthidine, pretazettine and tazettine from a common intermediate.



From **1.31** the first natural product, (±)-haemanthidine (**1.32**), was formed through reaction with phosphorus oxychloride followed by acetate deprotection. The second natural product, (±)-pretazettine (**1.33**), was formed from **1.32** by reaction of methyl iodide in methanol followed by treatment with acid. Methylation of the amine and opening of the hemiaminal was followed by ring closing to the lactol, giving **1.33**. For the third natural product, (±)-tazettine (**1.34**), **1.33** is treated with sodium hydroxide in

Scheme 1.7 Synthesis of (±)-crinamine B from common intermediate **1.30**.



methanol to give the hemiacetal through a Cannizzaro reaction.

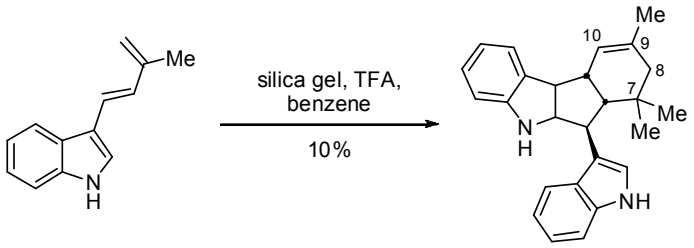
To access the fourth natural product, (±)-crinamine B (**1.36**), the path diverges from the other natural products at ketone **1.30** (Scheme 1.7). From **1.30**, a Luche reduction of the ketone followed by methylation of the resulting allylic alcohol gave **35**. Deprotection of the Boc and TMS groups followed by a Pictet-Spengler reaction with formaldehyde yields (±)-crinamine B. The syntheses of these four natural products are an example of how the Shapiro reaction employed early in synthetic routes can bring together large carbon frameworks that can be functionalized to provide a variety of different natural product targets from a common intermediate.

1. 5 Synthesis of Yuehchukene Analogues

Yuehchukene (**1.38**), an indole alkaloid natural product with anti-implantation activity, was first isolated in 1985; the first total synthesis was completed the same year.[11] The first total synthesis by Cheng was a biomimetic dimerization (Scheme 1.8); however

[11] Kong, Y.-C.; Cheng, K.-F.; Cambie, R. C.; Waterman, P. G. *J. Chem. Soc., Chem. Commun.* **1985**, 47-48.

Scheme 1.8 Cheng's biomimetic synthesis of yuehchukene.

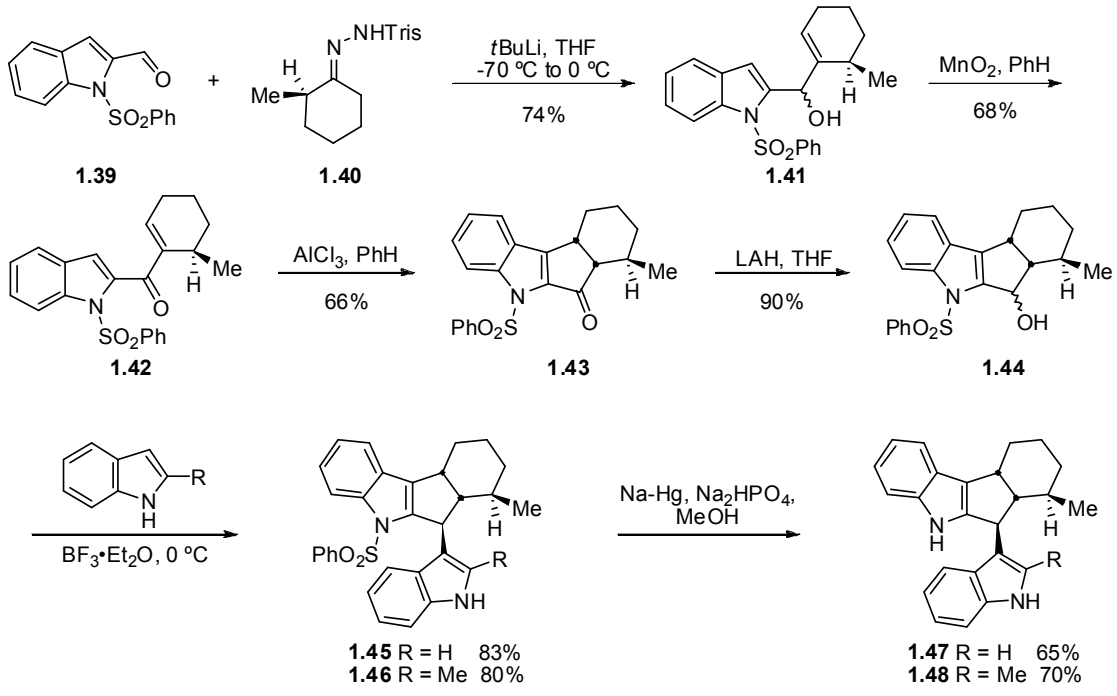


1.37 **1.38**

it suffered from the drawback that the dimerization step was low yielding.[12] In addition, attempting to synthesize analogues with alternate substituents at C7 was not possible with this route, with most attempts not giving any desired product.[13]

In order to access a series of analogues an early stage Shapiro reaction was

Scheme 1.9 Synthesis of two yuehchukene analogues.



1.39 **1.40** **1.41**

1.42 **1.43** **1.44**

1.45 R = H 83%
1.46 R = Me 80%

1.47 R = H 65%
1.48 R = Me 70%

[12] Cheng, K.-F.; Kong, Y.-C.; Chan, T.-Y. *J. Chem. Soc., Chem. Commun.* **1985**, 48-49.
[13] Cheng, K.-F.; Chan, T.-Y.; Wong, T.-T.; Lai, T.-F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 1555-1562.

utilized (Scheme 1.9).[14] Hydrazone **1.40** was accessed from the corresponding ketone, treated with *n*-BuLi and added to aldehyde **1.39**, giving **1.41** in 74% yield. This Shapiro reaction installed the carbon framework for the upper part of the molecule. The resulting secondary alcohol was obtained in a 1:1 diastereomeric ratio; however oxidation with manganese dioxide to ketone **1.42** eliminated the stereogenic center. This ketone underwent the key Nazarov cyclization to close the five-membered ring, resulting in **1.43**. The ketone was reduced with lithium aluminum hydride and the resulting alcohol was condensed with either indole or 2-methylindole, giving **1.45** or **1.46**, respectively. Removal of the phenyl sulfonyl protecting group with sodium amalgam gave **1.47** and **1.48**, both with the correct relative stereochemistry.

In addition to analogues **1.47** and **1.48**, Cheng and coworkers made an enantiomerically pure camphor derivative **1.50** and a simplified *t*-butyl analogue **1.51**.[15,16]

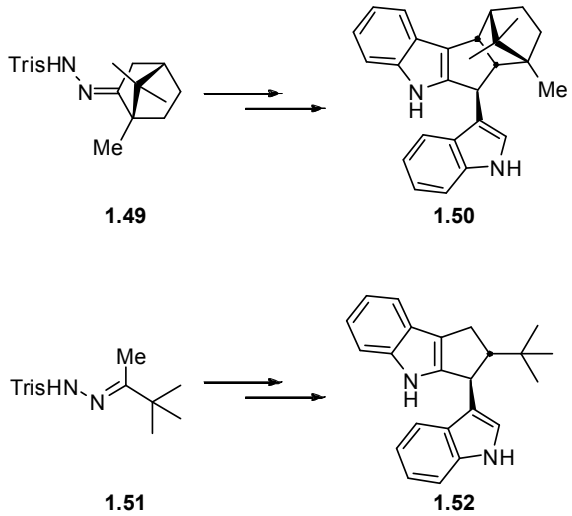


Figure 1.1 Yuehchukene analogues made via Shapiro coupling reactions.

[14] a) Cheng, K.-F.; Chan, K.-P.; *Synth. Commun.* **1990**, *20*, 3069-4076. b) Chan, K.-P.; Lai, T.-F. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 2461-2461.
[15] Cheng, K.-F.; Chan, K.-P.; Kong, Y.-C.; Ho, D.-D. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 2935-2939.

The syntheses of these analogues began with hydrazones **1.49** and **1.51**, respectively. They both follow a path nearly identical to that of analogues **1.47** and **1.48** with minimal changes of reagents.

From these investigations and SAR studies the Cheng group found that the simplified analogue **1.52** has comparable potency to both the natural product and the more complex analogues. In addition, the synthesis of analogue **1.50** helped to determine the absolute configuration of the natural product, showing similar potency while *ent*-**1.50** showed no activity. These studies show the generality of the Shapiro reaction and how it can be used to access a variety of related structures quickly and in good yields.

1.6 Synthesis of (-)-Ovalicin

Many groups have undertaken the synthesis of the natural product ovalicin, using a variety of synthetic methods such as cycloadditions[17] and carbohydrate chemistry.[18] A common method of installing the olefinic side chain uses the Shapiro reaction.[18,19] The efforts of the Samadi group, shown in Scheme 1.10, were the first to fully incorporate the coupling of the side chain directly from vinyl lithium **1.58**.[20]

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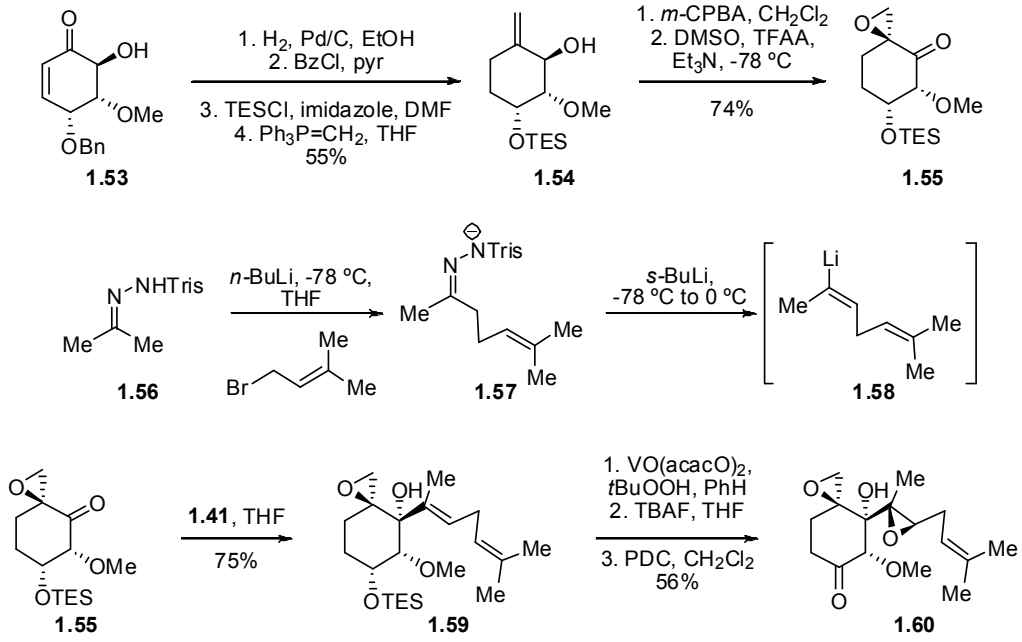
[16] Cheng, K.-F.; Cao, G.-A.; Yu, Y.-W. *Synth. Commun.* **1994**, *24*, 67-75.
[17] Tiefenbacher, K.; Arion, V. B.; Mulzer, J. *Angew. Chem. Int. Ed.* **2007**, *46*, 2690-2693.
[18] Yadav, J. S.; Sreedhar, P.; Srihari, P.; Sarma, G. D.; Jagadeesh, B. *Synthesis*, **2008**, 1460–1466.
[19] For other syntheses with similar incorporations of the olefinic side chain to that in Scheme 1.10 see: a) Corey, E. J.; Guzman-Perez, A.; Noe, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 12109-12110. b) Yamaguchi, J.; Toyoshima, M.; Shoji, M.; Kakeya, H.; Osada, H.; Hayashi, Y. *Angew. Chem. Int. Ed.* **2006**, *45*, 789-793. c) Hua, D. H.; Zhao, H.; Battina, S. K.; Lou, K.; Jimenez, A. L.; Desper, J.; Perchellet, E. M.; Perchellet, J.-P.; Chian, P. K. *Bioorg. Med. Chem.* **2008**, *16*, 5232-5246.
[20] a) Bath, S.; Billington, D. C.; Gero, S. D.; Quiclet-Sire, B.; Samadi, M. *J. Chem. Soc., Chem. Commun.* **1994**, 1495-1496. b) Barton, D. H. R.; Bath, S.; Billington, D. C.; Gero, S. D.; Quiclet-Sire, B.; Samadi, M. *J. Chem. Soc., Perkin Trans. 1*, **1995**, 1551-1558.

Scheme 1.10 Total synthesis of (-)-ovalicin employing a Shapiro reaction to append the side chain.



The synthesis began from **1.53**, which the Samadi group accessed from L-quebrachitol in 8 steps. The enone was hydrogenated, which also afforded benzyl deprotection. The more reactive α-alcohol was then benzoyl protected and the remaining free alcohol was protected as the TES ether. Wittig olefination of the ketone also resulted in benzoyl deprotection, giving **1.54**. Epoxidation of the exocyclic olefin with *m*-CPBA and oxidation of the free secondary alcohol gave the key intermediate **1.55**.

To generate the side chain, a protocol developed by the Corey group[21] was used. While the Corey group did use this synthon in the synthesis of ovalicin, they first trapped it with tributyltin chloride to give the vinyl stannane, which was then used in a separate step.

[21] a) Corey, E. J.; Dittami, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 256-257. b) Corey, E. J.; Lee, J.; Roberts, B. E. *Tetrahedron Lett.* **1997**, *38*, 8915-8918.

Acetone trisylhydrazone (**1.56**) was treated with two equivalents of *n*-BuLi and kept at -78 ºC to generate the diianion. The carbanion was reacted with prenyl bromide to give **1.57**. One equivalent of *s*BuLi was added to regenerate the carbanion and then the reaction was warmed to 0 ºC to facilitate loss of nitrogen and generate the vinyl lithium **1.58**.

Keto epoxide **1.55** was then added to vinyl lithium **1.58**. Approach of the nucleophile from the less hindered face gave tertiary alcohol **1.59** as the sole product in 75% yield. Epoxidation of the side chain was followed by deprotection of the TES group and oxidation with PDC gave the natural product (-)-ovacilin, **1.60**. This synthesis highlights the ability of the Shapiro reaction in constructing sterically hindered molecules through the generation of a quaternary center.

1.7 Improved Access to the Ingenol Core

To date there have been three total syntheses of the natural product ingenol (**1.61**) by Winkler,[22] Kuwajima[23] and Wood.[24] While these syntheses are very impressive for the efforts they entailed, they all have one common thread; all three are long, linear syntheses ranging from 35 to 46 steps.[25]

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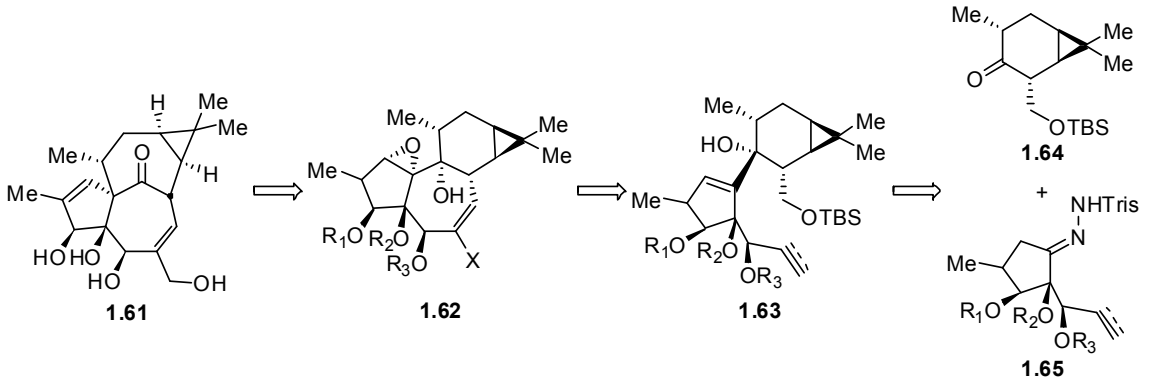
[22] Winkler, J. D.; Rouse, M. B.; Greaney, M. F.; Harrison, S. J.; Jeon, Y. T. *J. Am. Chem. Soc.* **2002**, *124*, 9726-9728.
[23] Tanino, K.; Onuki, K.; Asano, K.; Miyashita, M.; Nakamura, T.; Takahashi, Y.; Kuwajima, I. *J. Am. Chem. Soc.* **2003**, *125*, 1498-1500.
[24] Nickel, A.; Maruyama, T.; Tang, H.; Murphy, P. D.; Greene, N. Y.; Wood, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 16300-16301.
[25] For a review of these syntheses and other approaches, see: Cha, J. K.; Epstein, O. L. *Tetrahedron*, **2006**, *62*, 1329-1343.

Scheme 1.11 The Cha group's Retrosynthesis of ingenol.



The Cha group developed a convergent approach to a simplified ingenol core with intentions of elaborating their work to a total synthesis (Scheme 1.11).[26] Retrosynthetically, ingenol can be simplified to **1.62** (where X is an alkyl or alkenyl group) through a 1,2-alkyl shift. In turn, **1.62** can come from **1.63** after removal of the TBS group, oxidation and olefination followed by ring closing olefin or enyne metathesis. The coupling of two advanced fragments, **1.64** and **1.65** through a Shapiro reaction will help to quickly construct the core.

In order to rapidly determine the strength of their retrosynthetic analysis, the Cha group decided to test their route with a simplified variant of **1.65** in a Shapiro reaction with **1.64** (Scheme 1.12). Starting from ketone **1.66**[27] deprotonation and trap with methyl iodide led to **1.64**. A Shapiro reaction with simplified coupling partner **1.67** provided the coupled product **1.68** in 80% yield and a three step sequence of TBAF deprotection, oxidation and olefination gave the diene **1.69**. A ring closing metathesis successfully generated **1.70**, which is close to providing the simplified core.

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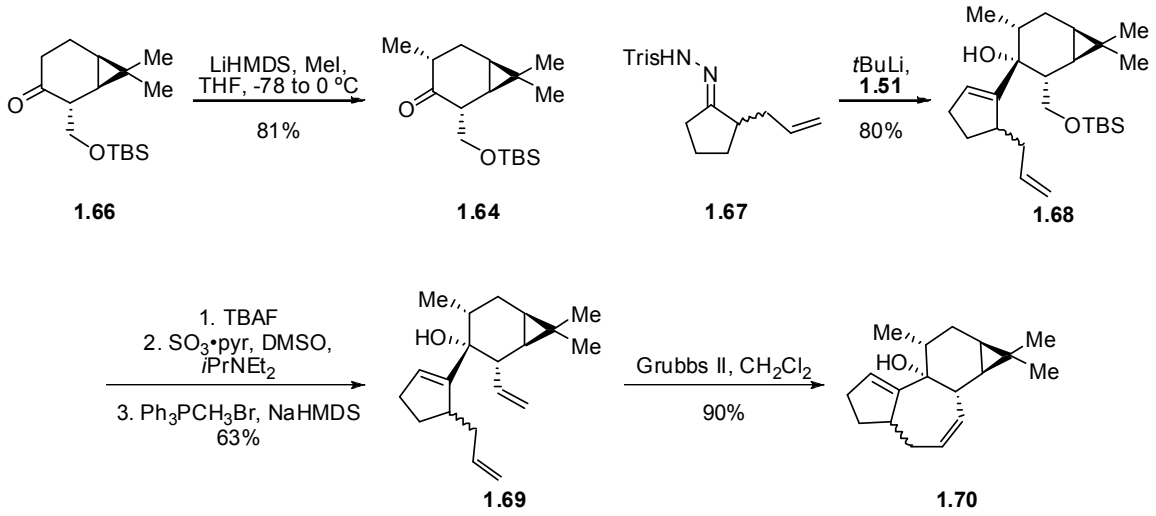
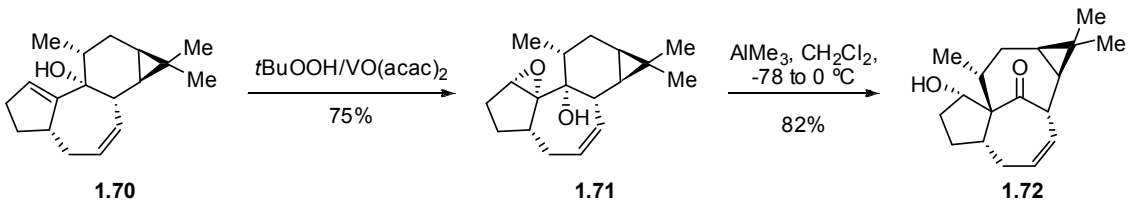
[26] Epstein, O. L.; Cha, J. K.; *Angew. Chem. Int. Ed.* **2005**, *44*, 121-123.
[27] For preparation of **1.49** from (+)-3-carene, see: Paquette, L. A.; Ross, R. J.; Shi, Y. S. *J. Org. Chem.* **1990**, *55*, 1589-1598.

Scheme 1.12 Cha's test substrate approach to the ingenol core.



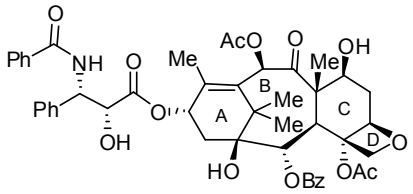
Completing the synthesis of the core required separating the diastereomers of **1.70**. Once a diastereomerically pure **1.70** was accessed, two steps transformed it into the core structure **1.72** (Scheme 1.13). Olefin **1.70** was first treated with *t*-BuOOH-$VO(acac)_2$ providing the regioselectively epoxidized **1.71**, which was then treated with $AlMe_3$ at low temperatures inducing the 1,2-alkyl shift. **1.72** is a simplified version of the ingenol core, **1.62**. Structures similar to **1.62** have previously been elaborated to ingenol. With the proper coupling partners, the Shapiro reaction has the potential to drastically shorten the route to ingenol.

Scheme 1.13 Completion of the simplified ingenol core.



1.8 Nicolaou's Total Synthesis of Taxol

Taxol (**1.73**), a diterpene isolated from the bark of the pacific yew tree, is a very important natural product in the area of cancer therapy. It has been in use in the US as a breast and ovarian cancer drug since 1993.[28] In the late 1980s and early 1990s many laboratories were active in trying to achieve the first total synthesis of Taxol. In 1994 Holton and Nicolaou published almost simultaneous reports. The Holton group published a linear approach starting from patchino.[29]



1.73

The Nicolaou group approached Taxol in a convergent fashion, with the intention of connecting the A and C rings and then forming the B ring, followed by later installation of the D ring and the side chain.[30]

Nicolaou's synthetic efforts toward the A ring began with converting **1.74** into diene **1.76** for use in a Diels-Alder reaction (Scheme 1.14). Ketone **1.74** underwent methyl Grignard addition followed by elimination of the resulting tertiary alcohol gave 1,1-disubstituted olefin **1.75**. The ethyl ester **1.75** was subjected to DIBAL reduction and acetate protection of the ensuing primary alcohol. With diene **1.76** in hand, a Diels-Alder cycloaddition with 2-chloroacrylonitrile gave **1.77** with the desired regiochemistry. The

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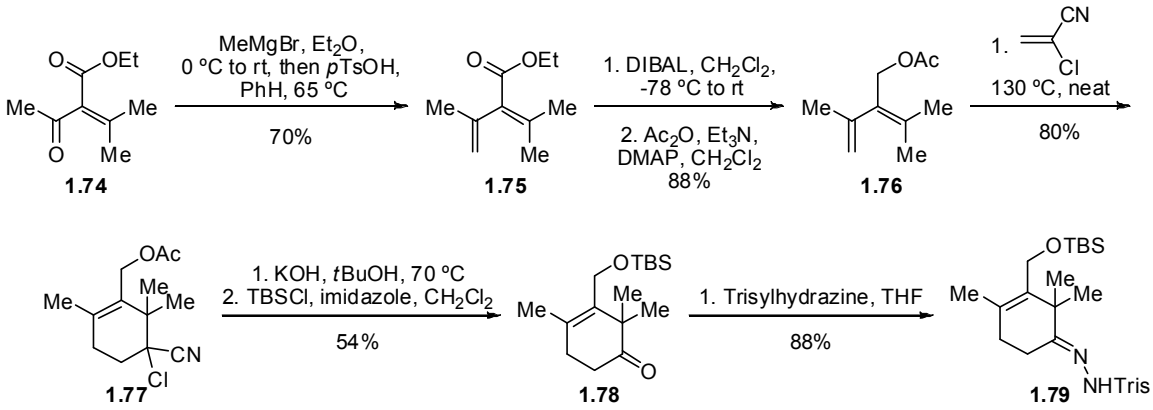
[28] Rowinksy, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. *Semin. Oncol.* **1992**, *19*, 646-662.
[29] Holton, R. A.; Somoza, C.; Kim, H-B.; Liang. F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1597-1598.
[30] a) Nicolaou, K. C.; Nantermet, P. G.; Ueno, H.; Guy, R. K.; Couladouros, E. A.; Sorensen, E. J. *J. Am. Chem. Soc.* **1995**, *117*, 624-633. b) Nicolaou, K. C.; Liu, J.-J. Yang, Z.; Ueno, H.; Sorensen, E. J.; Claiborne, C. F.; Guy, R. K.; Hwang, C.-K.; Nakada, M.; Nantermet, P. G. *J. Am. Chem. Soc.* **1995**, *117*, 634-544. c) Nicolaou, K. C.; Yang, Z.; Liu, J.-J.; Nantermet, P. G.; Claiborne, C. F.; Renaud, J.; Guy, R. K.; Shibayama, K. *J. Am. Chem. Soc.* **1995**, *117*, 645-652. d) Nicolaou, K. C.; Ueno, H.; Liu, J.-J.; Nantermet, P. G.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chadha, R. *J. Am. Chem. Soc.* **1995**, *117*, 653-659.

Scheme 1.14 Nicolaou's synthesis of the A ring of Taxol.



acetate group of **1.73** was removed and replaced with a TBS group and the ketone was converted to the trisylhydrazone. This completed the synthesis of the A ring synthon and sets up a Shapiro reaction to couple ring A to ring C.

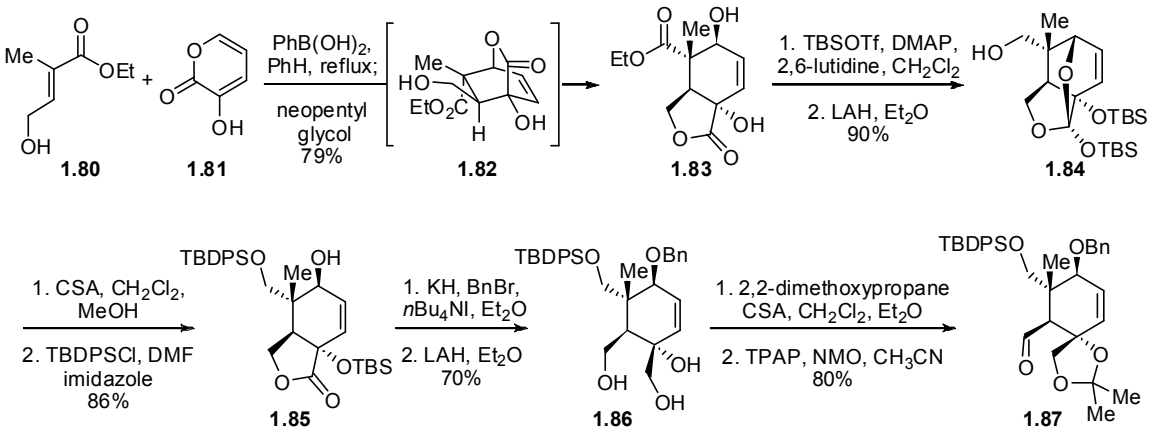
Prior to deciding on the Shapiro reaction as a coupling strategy the Nicolaou group also looked into using a Stille coupling. While they were able convert ketone **1.78** into a vinyl triflate and then the vinyl stannane, they were not successful with coupling to an appropriately functionalized C ring. Simple couplings, such as with benzoyl chloride gave positive results; therefore, it was postulated that the steric hindrance of the gem-dimethyl group on **1.79** impeded the Stille coupling with more sterically demanding substrates. Therefore, Nicolaou decided on the Shapiro reaction for coupling the two ring systems and set out to synthesize a suitably functionalized C ring.

To begin the synthesis of the C ring, a Diels-Alder reaction between **1.80** and pyrone **1.81** was performed (Scheme 1.15). To overcome the inherent regioselectivity of the reaction, Nicolaou employed a procedure developed by the Narasaka group that

temporarily tethers the allylic alcohol of **1.80** and the enol ether of **1.81**.[31] This temporary tether gave the regioselectivity shown in **1.82** after decomplexation of the boronic ester with neopentyl glycol.

The strained **1.82** spontaneously rearranged under the reaction conditions, giving **1.83**. Attempted protection of the free alcohols with TBSOTf resulted in addition of the secondary alcohol to the lactone followed by protection of the tertiary alcohol and the new orthoester.[32] Reduction of the ethyl ester with lithium aluminum hydride gave the primary alcohol **1.84**. Treatment of **1.84** with CSA deprotected the lactol TBS group and gave back the lactone and free secondary alcohol. TBDPS protection of the primary alcohol gave **1.85**. Benzyl protection of the secondary alcohol was followed by exhaustive reduction of the lactone with concomitant deprotection of the TBS group to give triol **1.86**. The triol was treated with CSA and 2,2-dimethoxypropane to initially

Scheme 1.15 Nicolaou's synthesis of a C ring synthon.

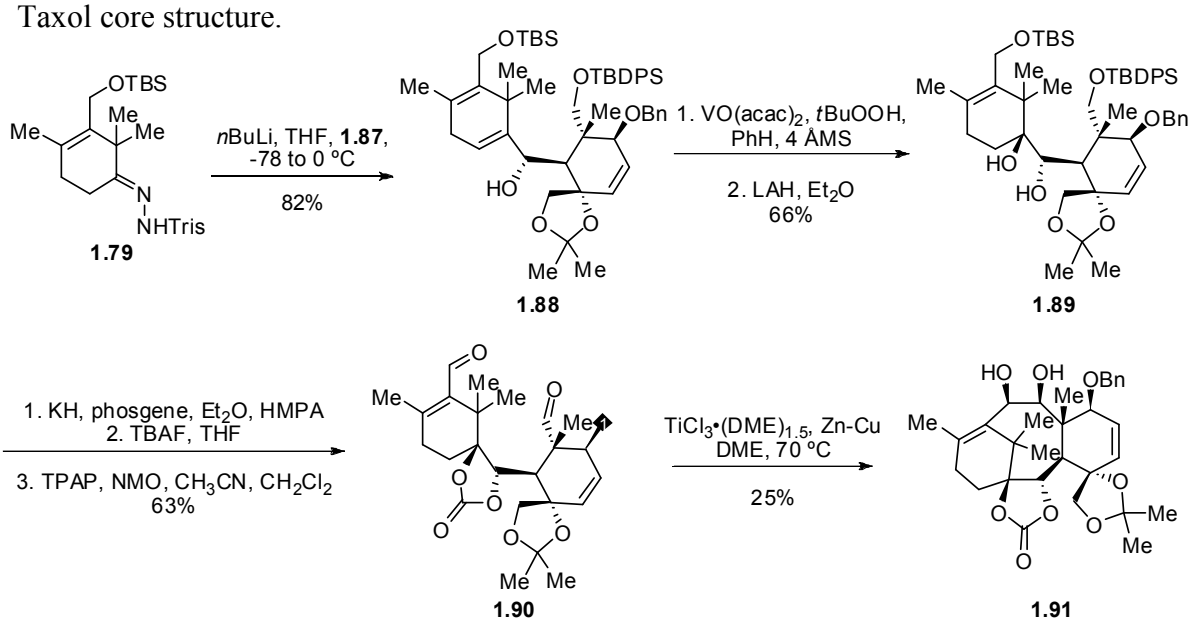


[31] Narasaka, K.; Shimada, S.; Osoka, K.; Iwasawa, N. *Synthesis* **1991**, 1171-1172.
[32] For a correction on the structure from the original paper see: Nicolaou, K. C.; Liu, J.-J.; Yang, Z.; Ueno, H.; Sorensen, E. J.; Claiborne, C. F.; Guy, R. K.; Hwang, C.-K.; Nakada, M.; Nantermet, P. G. *J. Am. Chem. Soc.* **1995**, *117*, 8690.

give a seven-membered acetal incorporating the two primary alcohols; however, after extended exposure to the reaction conditions rearrangement to the more thermodynamically stable five-membered acetal took place. Oxidation of the remaining free alcohol to the aldehyde with TPAP/NMO gave aldehyde **1.87**, the C ring synthon necessary for a Shapiro coupling with the A ring synthon.

To couple **1.79** and **1.87** through a Shapiro reaction **1.79** was first treated with *n*-BuLi generating the vinyl anion (Scheme 1.16). Aldehyde **1.87** was added, giving the secondary alcohol **1.88**. The resulting diastereoselectivity is hypothesized to come from a lithium chelate between the aldehyde and acetal, which blocks *re* face addition of carbanion nucleophile. From **1.88** the new trisubstituted olefin was epoxidized diastereoselectively and the epoxide opened with lithium aluminum hydride to give tertiary alcohol **1.89**. This new diol was protected as the carbonate upon treatment with KH and phosgene. The two silyl groups were deprotected and the resulting primary alcohols were oxidized with TPAP/NMO, giving dialdehyde **1.90**. The key McMurray coupling of **1.90** using titanium trichloride and zinc-copper couple gave an optimized yield of 25% for diol **1.91,** completing the synthesis of the core structure of Taxol. This core structure was elaborated to the natural product (**1.73**) in 17 steps.[30d] The coupling of these two fragments highlights the utility of the Shapiro reaction in sterically hindered settings where transition-metal mediated couplings are not successful.

Scheme 1.16 Shapiro coupling between A and C ring synthons and completion of the Taxol core structure.



1.9 Total Synthesis of (-)-Colombiasin A and (-)-Elisapterosin B

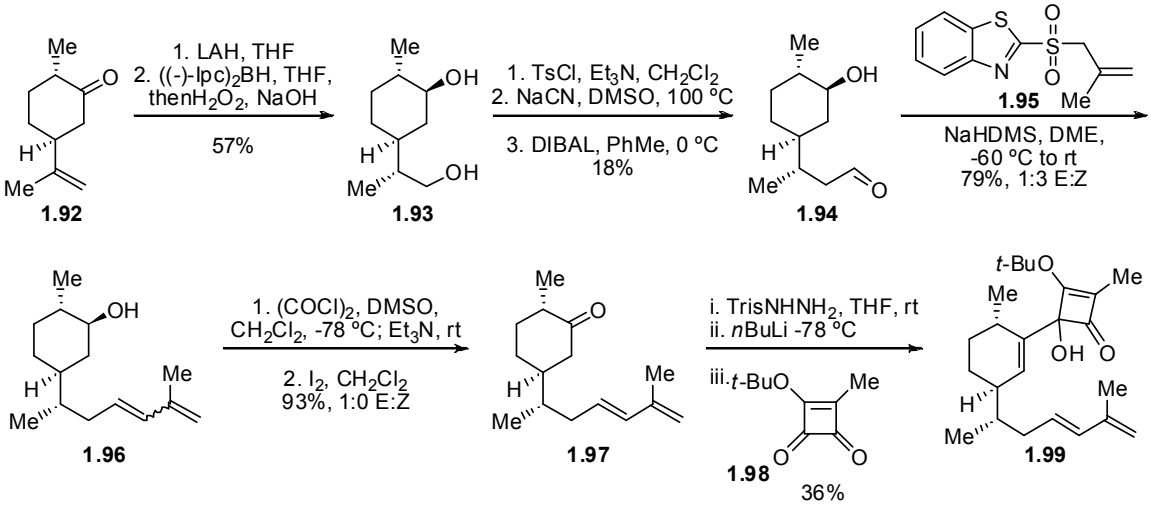
(-)-Colombiasin A (**1.105**) and (-)-elisapterosin B (**1.103**) (Scheme 1.18) were isolated from *Pseudopterogorgia elisabethae* and quickly became of interest to the synthetic community. Several total syntheses have been completed since 2001.[33] In 2005 the Harrowven group approached the synthesis of these two molecules in a convergent fashion, using a Shapiro reaction to bring together two fragments in preparation for a key Moore rearrangement.[34]

The synthesis began with the reduction of ketone **1.92** (Scheme 1.17) with LAH

[33] a) Nicolaou, K. C.; Vassilikogiannakis, G.; Mägerlein, W.; Kranich, R. *Angew. Chem. Int. Ed.* **2001**, *40*, 2482-2486. b) Nicolaou, K. C.; Vassilikogiannakis, G.; Mägerlein, W.; Kranich, R. *Chem. Eur. J.* **2001**, *7*, 5359-5371. c)Kim, A. I.; Rychnovsky, S. D. *Angew. Chem. Int. Ed.* **2003**, *42*, 1267-1270. d) Waizumi, N.; Stankovic, A. R.; Rawal, V. H. *J. Am. Chem. Soc.* **2003**, *125*, 13022-13023. e) Boezio, A. A.; Jarvo, E. R.; Lawrence, B. M.; Jacobsen, E. N. *Angew. Chem. Int. Ed.* **2005**, *44*, 6046-6050. f) Davies, H. M. L.; Dai, X.; Long, M. S. *J. Am. Chem. Soc.* **2006**, *128*, 2485-2490.

[34] Harrowven, D. C.; Pascoe, D. D.; Demurtas, D.; Bourne, H. O. *Angew. Chem. Int. Ed.* **2005**, *44*, 1221-1222.

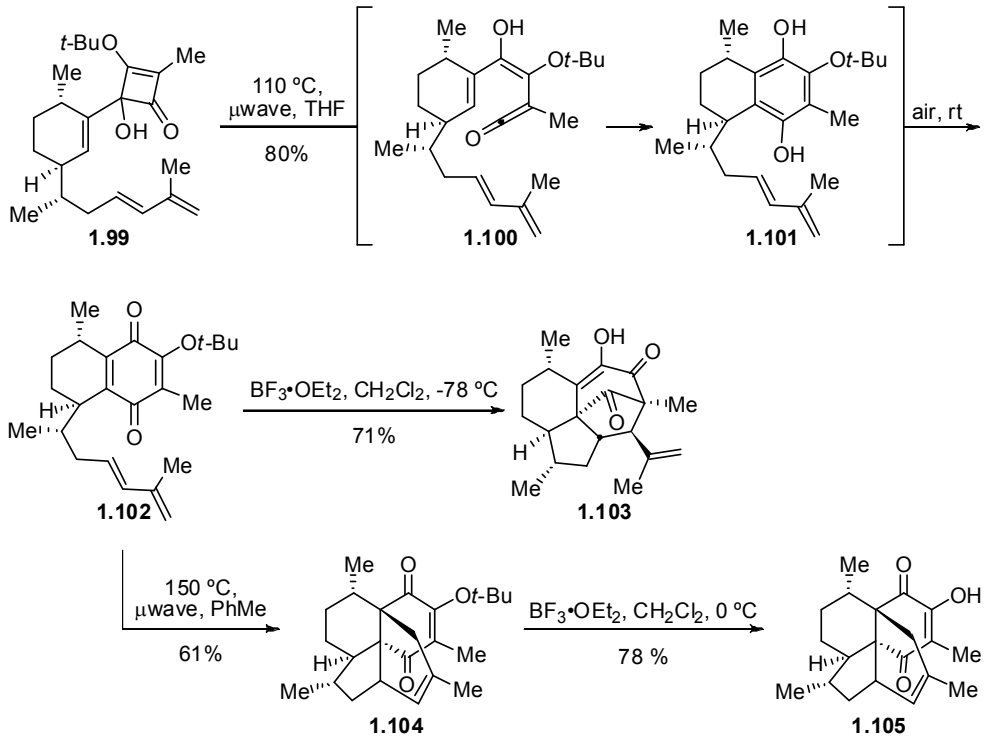
Scheme 1.17 Shapiro coupling and preceding steps in Harrowven's synthesis of (-)-colombiasin A and (-)-elisapterosin B.



followed by hydroboration of the 1,1-disubstituted olefin, giving diol **1.93**. The primary alcohol was converted to the tosylate, which was then displaced with sodium cyanide. The nitrile was reduced to the aldehyde with DIBAL, giving homologated product **1.94**. The aldehyde was subjected to a Julia-Kocienski olefination, yielding the new 1,1-disubstituted olefin **1.96** in a 1:3 ratio of E:Z isomers which were carried forward as a mixture. The secondary alcohol was then reoxidized to the ketone and the E:Z isomers were resolved through equilibration with iodine giving the desired E isomer **1.97** exclusively.

The authors then wanted to append the squarate **1.98** to the cyclohexene moiety using a Shapiro reaction; however, isolation of the trisylhydrazone of **1.97** was not possible. It was found that although they could form the hydrazone, it decomposed upon standing at room temperature. Generation of the trisylhydrazone and then cooling of the reaction mixture followed by addition of *n*-BuLi and then squarate **1.98** gave a 36% yield

Scheme 1.18 Completion of the syntheses of (-)-colombiasin A and (-)-elisapterosin B via a Moore rearrangement.



of the desired tertiary alcohol **1.95** as a mixture of diastereomers, along with some of the proton quenched product.

With the coupled product in hand, the key Moore rearrangement was investigated (Scheme 1.18). Squarate **1.99** was heated to 110 ºC under microwave heating conditions, giving an elecrocyclic ring opening to ketene **1.100**. This ketene in turn underwent an electrocylization followed by tautomerization, yielding bis-phenol **1.101**. Upon exposure to air **1.102** was oxidized to quinone **1.102**, in 80% yield from **1.99**. Treatment of **1.102** with borontrifluoride etherate removed the *t*Bu protecting group and induced a [5+2] cycloaddition, giving (-)-elisapterosin B (**1.103**). Quinone **1.102** can also be heated to 150 ºC under microwave heating, giving Diels-Alder product **1.104**, which was converted

to (-)-colombiasin A (**1.105**) after removal of the *t*Bu protecting group with borontrifluoride etherate.

These syntheses are a good example of how a Shapiro reaction can be utilized later in the synthetic pathway to bring together larger fragments, providing a product that has all of the necessary carbons in place. This allows for a rapid increase in complexity in the synthetic scheme, one of the most beneficial aspects of a convergent synthesis.

1.10 Synthesis of the AB Ring Fragment of Spongistatin 1

Spongistatin 1 (**1.106**) is an complex natural product comprised of a 42-membered macrolide framework with two spiroketals. To date a variety of groups have worked on the total synthesis of this molecule and also that of spongistatin 2 (dechloro-spongistatin 1).[35] The Smith group published an initial total synthesis in 2001,[36] and in 2002 followed up with an improved synthesis of the ABCD ring fragment.[37]

Retrosynthetic analysis of spongistatin (Scheme 1.19) shows that the natural product can be split roughly in half through an olefination and a macrolactonization. The ABCD ring fragment section in turn can be broken down further through an aldol

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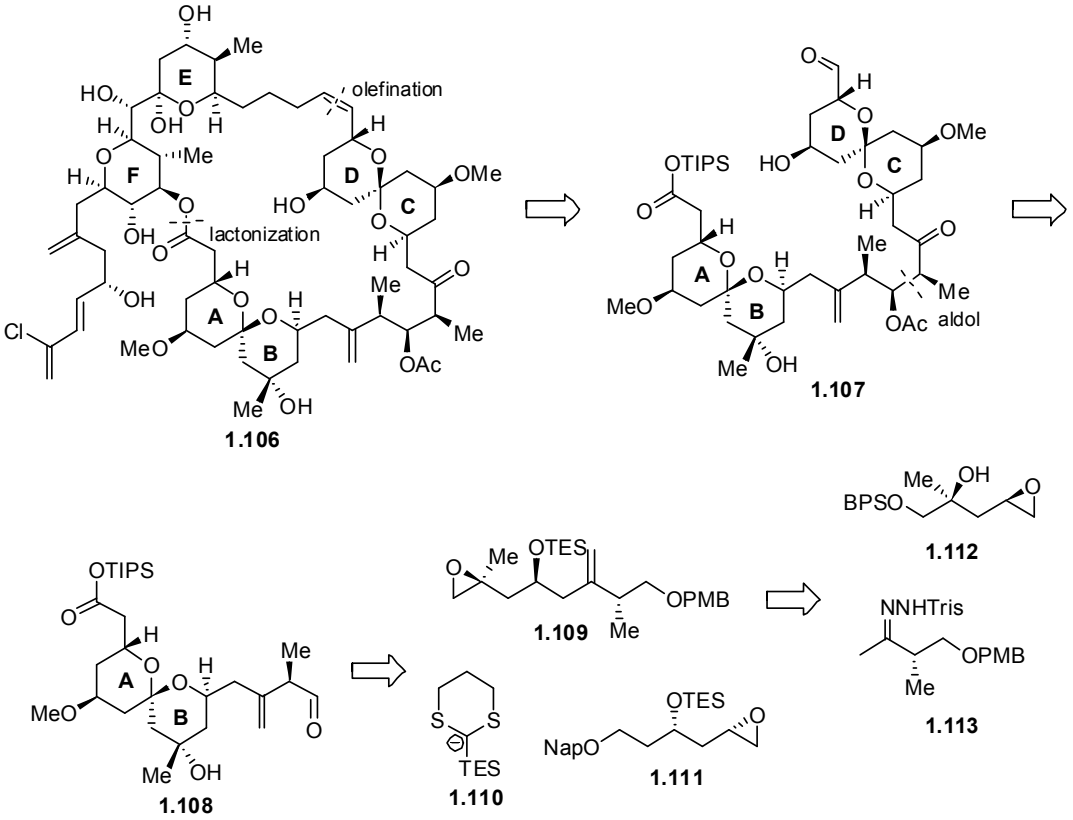
[35] a) Hayward, M. M.; Roth, R.; Duffy, K. J.; Dalko, P. I.; Stevens, K. L.; Guo, J.; Kishi, Y. *Angew. Chem. Int. Ed.* **1998**, *37*, 192-196. b) Evans, D. A.; Trotter, B. W.; Côté, B.; Coleman, P. J.; Dias, L. C.; Tyler, A. N. *Angew. Chem. Int. Ed.* **1997**, *36*, 2744-2747. c) Paterson, I.; Chen, D. Y.-K.; Coster, M. J.; Aceña, J. L.; Bach, J.; Gibson, K. R.; Keown, L. E.; Oballa, R. M.; Trieselmann, T.; Wallace, D. J.; Norcross, R. D. *Angew. Chem. Int. Ed.* **2001**, *40*, 4055-4060.

[36] a) Smith, A. B.; Doughty, V. A.; Lin, Q.; Zhuang, L.; McBriar, M. D.; Boldi, A. M.; Moser, W. H.; Murase, N.; Nakayama, K.; Sobukawa, M. *Angew. Chem. Int. Ed.* **2001**, *40*, 191-195. b) Smith, A. B.; Lin, Q.; Doughty, V. A.; Zhuang, L.; McBriar, M. D.; Kerns, J. K.; Brook, C. S.; Murase, N.; Nakayama, K. *Angew. Chem. Int. Ed.* **2001**, *40*, 196-199.

[37] Smith, A. B.; Doughty, V. A.; Sfouggatakis, C.; Bennet, C. S.; Koyanagi, J.; Takeuchi, M. *Org. Lett.* **2002**, *4*, 783-786.

Scheme 1.19 Retrosynthesis of Smith's second generation synthesis of spongistatin 1.

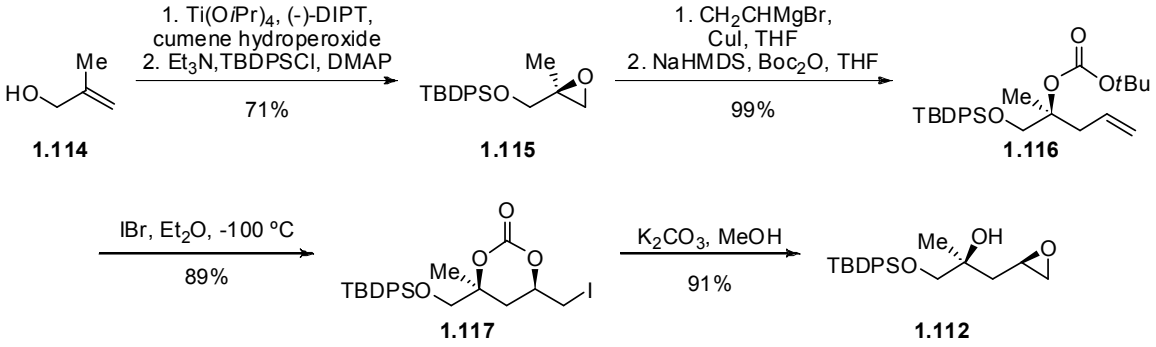


disconnection. The AB ring fragment is further divided with a three component coupling reaction between **1.109**, **1.110** and **1.111**. Epoxide **1.109** is provided via Shapiro reaction of **1.112** and **1.113**.

To begin the synthesis of the AB ring fragment (Scheme 1.20), allylic alcohol **1.114** underwent a Sharpless asymmetric epoxidation followed by protection of the primary alcohol, giving **1.115**. Opening of the epoxide with a vinyl cuprate and protection of the resulting secondary alcohol as the Boc carbonate gave **1.116**. Iodocarbonate cyclization of **1.116** led to **1.117** with good *syn* selectivity. Methanolysis gave deprotection of the carbonate and concomitant epoxide formation, providing **1.112**.

To continue the synthesis, fragment **1.120** was synthesized starting from methyl

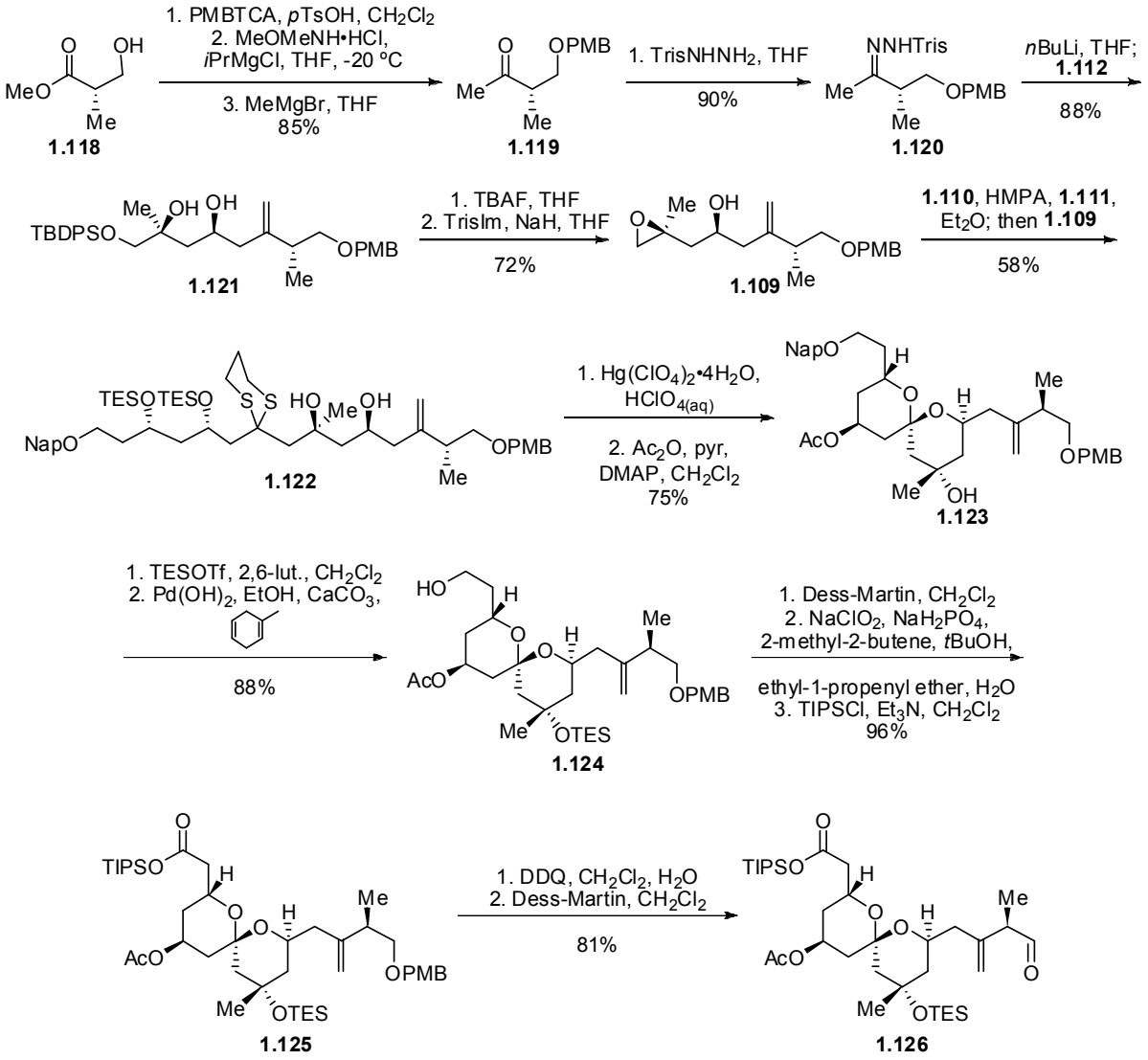
Scheme 1.20 Synthesis of epoxide **1.112** for the AB ring fragment of spongistatin.



ester **1.118** (Scheme 1.21). PMB protection of the primary alcohol followed by Weinreb amide formation and methyl Grignard addition gave methyl ketone **1.119**. This ketone was transformed in good yield to the trisylhydrazone **1.120** in preparation for a Shapiro coupling. The trisylhydrazone was treated with *n*-BuLi and then epoxide **1.112** was added, giving diol **1.121** in good yield. Deprotection of the TBDPS group followed by epoxide formation gave key intermediate **1.109**.

Next in the synthetic pathway was the key three component coupling. Lithiated TES-dithiane **1.110** was added to epoxide **1.111**. A Brooke rearrangement ensued and then epoxide **1.109** was added, resulting in diol **1.122**. Deprotection of the dithiane protecting group also served to deprotect the TES groups and gave the spiroketalization necessary for the AB ring system. This product was then treated with acetic anhydride to protect the free secondary alcohol, giving **1.123**. The tertiary alcohol was protected as the TES ether and a transfer hydrogenolysis removed the naphthyl protecting group on the primary alcohol, providing **1.124**. Oxidation of the free alcohol to the aldehyde with Dess-Martin periodinane was followed by further oxidation to the carboxylic acid. TIPS protection of the acid gave **1.125**. Deprotection of the PMB group and oxidation to the

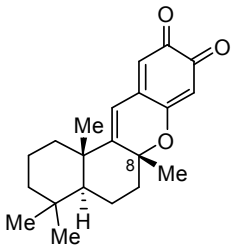
Scheme 1.21 Completion of the AB ring fragment of spongistatin.



aldehyde gave the AB ring fragment **1.126**, which is identical to the fragment used in Smith's earlier total synthesis of spongistatin. Use of the Shapiro reaction to form this fragment allowed for the synthesis of the ABCD ring fragment in 15 fewer steps than in the previous synthesis.

1.11 Formal Total Synthesis of 8-Epipuupewhedione from (*R*)-(-)-Carvone

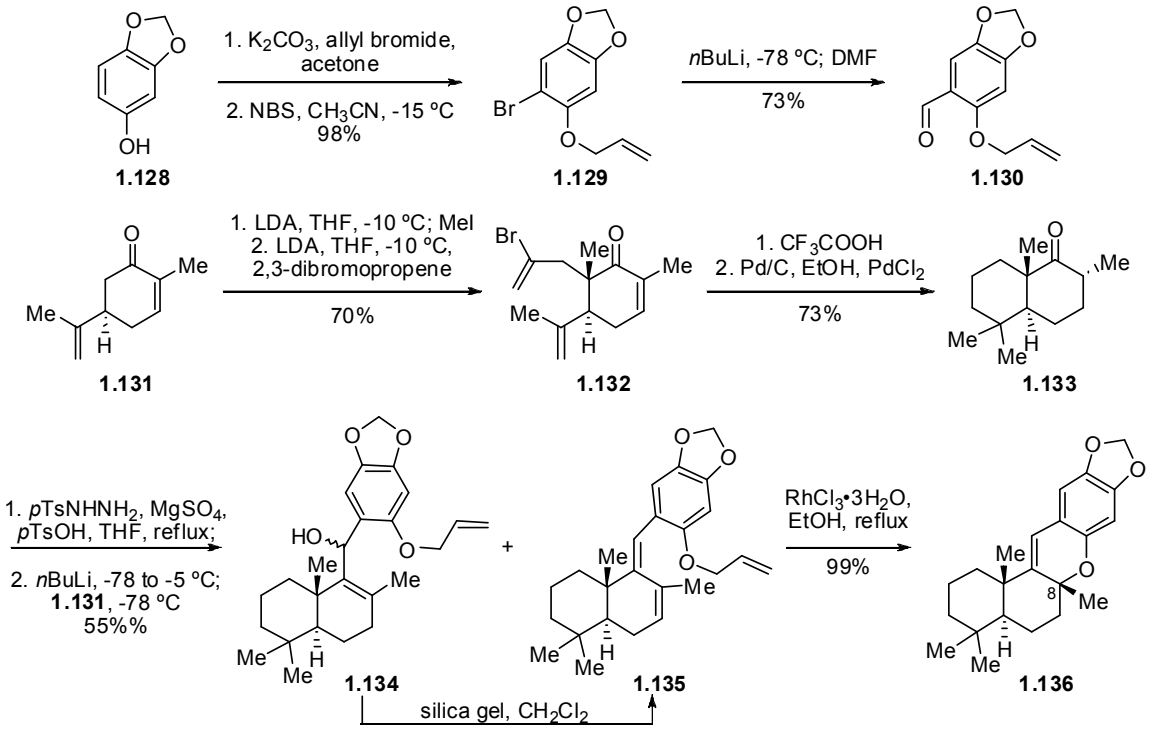
8-epipuuphehedione (**1.127**) is the C8 epimer of puuphehedione, a marine sponge metabolite. The C8 epimer displays a greater degree of activity against tumor cell lines 388, A-549, HT-29 and MEL-28.[38] The Banerjee group reported a formal total synthesis of 8-epipuuphehedione from (*R*)-(-)-carvone (**1.131**), utilizing a late-stage Shapiro reaction.[39]



1.127

The total synthesis began with the synthesis of the aromatic portion of the molecule (Scheme 1.22). Starting from the commercially available sesamol (**1.128**),

Scheme 1.22 Banerjee's total synthesis of 8-epipuuphehedione.



[38] a) Barrero, A. F.; Alvarez-Manzandeda, E. J.; Chahboun, R. *Tetrahedron Lett.* **1997**, *38*, 2325-2328. b) Barrero, A. F. Alvarez-Manzandeda, E. J.; Chahboun, R.; Cortés, M.; Armstrong, V. *Tetrahedron*, **1999**, *55*, 15181-15208.

allylation and bromination gave **1.129** in excellent yield. Bromide **1.129** was converted to aldehyde **1.130** by lithium halogen exchange and trap with DMF in preparation for the Shapiro coupling.

To assemble the decalin portion of the molecule, **1.131** was treated with LDA and the resulting enolate trapped with methyl iodide. This new compound was again treated with LDA and the enolate trapped with 2,3-dibromopropane, giving **1.132** diastereoselectively. Treatment of the vinyl bromide with trifluoroacetic acid gave the decalin system as a mixture of olefin regioisomers. This mixture of olefin isomers underwent a palladium catalyzed hydrogenation, which also reduced the α,β-unsaturated olefin and the bromide. Ketone **1.133** was then transformed into the tosylhydrazone in good yield and treated with *n*-BuLi at low temperatures to generate the vinyl anion. Aldehyde **1.130** was added and the reaction yielded a mixture of unstable allylic alcohol **1.134** and desired diene **1.135**. The allylic alcohol can be transformed into the diene through treatment with silica gel in methylene chloride, giving **1.135** in good yield from the hydrazone. Treatment of **1.135** with rhodium trichloride removed the allyl protection group and induced cyclization to **1.136**, which constitutes a formal total synthesis of 8-epipuupehedione. The natural product can be accessed in one step from **1.136** by oxidation with DDQ and TsOH in refluxing dioxane.[38b] This synthesis exemplifies the use of the Shapiro reaction as a late stage coupling tool. Both ring fragments are highly functionalized and once brought together the synthesis is nearly complete.

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[39] Maiti, S.; Sengupta, S.; Gtiri, C.; Achari, B.; Banerjee, A. K. *Tetrahedron Lett.* **2001**, *42*, 2389-2391.

1.12 Conclusion

These fifteen examples of the Shapiro reaction being used to couple two fragments of a molecule during natural product total synthesis have shown a wide range of utility for this reaction. Used early in the synthesis, the Shapiro reaction can easily bring together two simple fragments that can be elaborated into a vast array of final structures. Used later in the synthesis a Shapiro reaction can bring together more advanced intermediates, often setting up the key step of the synthesis. Used at the end of the synthesis, the Shapiro reaction allows for two highly functionalized synthons to be joined with high functional group compatibility.

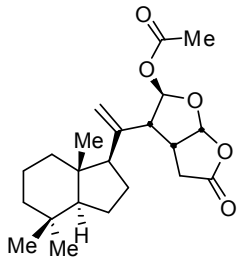
In all, the Shapiro reaction is often overlooked in favor of the more popular transition metal-mediated coupling reactions; however, it is a simple and effective method of coupling molecules together, especially in the context of natural product total synthesis.

Chapter 2

Total Synthesis of Norrisolide

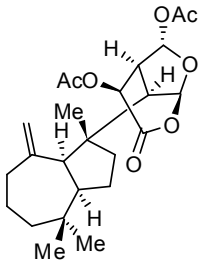
2.1 Introduction

Norrisolide (**2.1**) is a marine natural product that was isolated from the nudibranch mollusc *Chromodoris norrisi* in 1983.[1] Structural determination was done by X-ray crystallography and proton NMR experiments. Norrisolide has also been isolated as a minor component from other nudibranch molluscs. Along with norrisolide, three other compounds were isolated from *Chromodoris norrisi*: macfarlandin E (**2.2**), polkyhaphin A (**2.3**) and shahamin C (**2.4**) (Figure 2.1).[2]

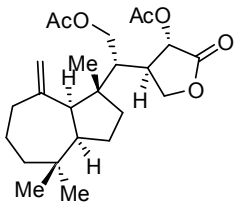


2.1

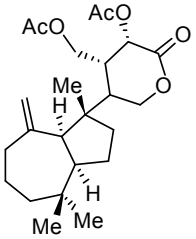
In addition to being isolated from molluscs, norrisolide has also been isolated in minor amounts from sponges in the same area. In light of this fact, it has been



2.2



2.3



2.4

Figure 2.1 Other compounds isolated from *Chromodoris norrisi*.

[1] Faulkner, D. J.; Hochlowski, J. E. *J. Org. Chem.* **1983**, *48*, 1141-1142.
[2] Bozbin, S. C.; Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 3902-3907.

hypothesized that the molluscs acquire the precursor to norrisolide and related compounds through feeding on the sponges.[3] The general precursor to these diterpenes is believed to be the unknown compound spongiane (**2.5**). More evidence supporting this theory is that several compounds related to the spongiane skeleton have been isolated from marine sponges that are taxonomically similar to those that have been shown to contain rearranged diterpene natural products. Examples of these compounds[4] are shown in Figure 2.2 and include aplysillin[5] (**2.6**), isoagotholactone[6] (**2.7**), aplyroseol-1 (**2.8**), aplyroseol-14 (**2.9**), aplyroseol-16[7] (**2.10**) and spongian-16-one[8] (**2.11**).

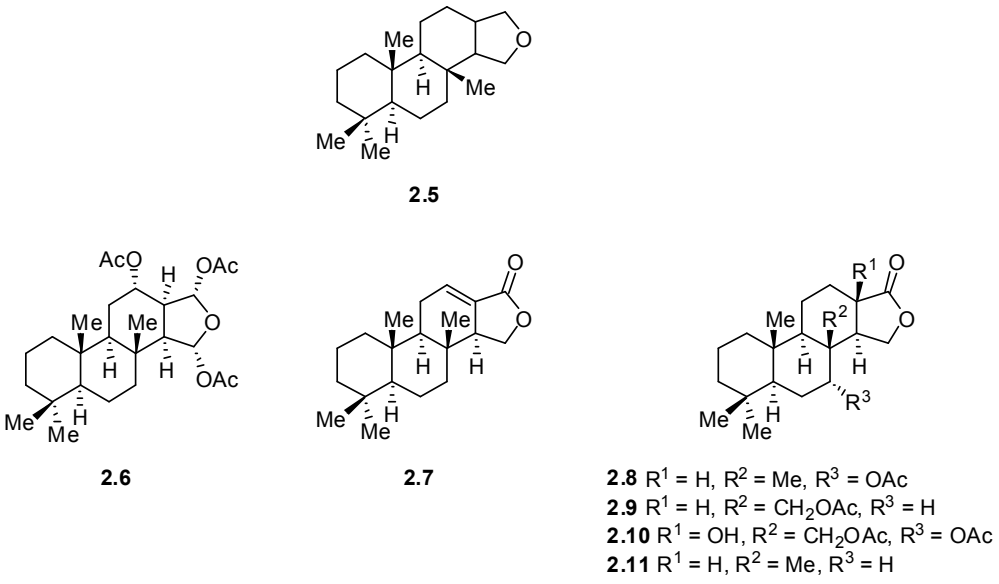


Figure 2.2 Compounds containing the spongiane skeleton.

[3] Carmely, S.; Cojocaru, Y. L.; Kashman, Y. *J. Org. Chem.* **1988**, *53*, 4801-4807.
[4] For a review of spongiane diterpenoids in both intact and rearranged forms see: González, M. A. *Curr. Bioact. Compd.*, **2007**, *3*, 1-36.
[5] Kazlaukas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1979**, 903-906.
[6] Cimino, G.; De Rosa, D.; De Stefano, S.; Minale, L. *Tetrahedron* **1974**, *30*, 645-649.
[7] Taylor, W.; Toth, S. *Aust. J. Chem.* **1997**, *50*, 895-902.
[8] Kernan, M. R.; Cambie, R. C.; Bergquist, P. R. *J. Nat. Prod.* **1990**, *53*, 724-727.

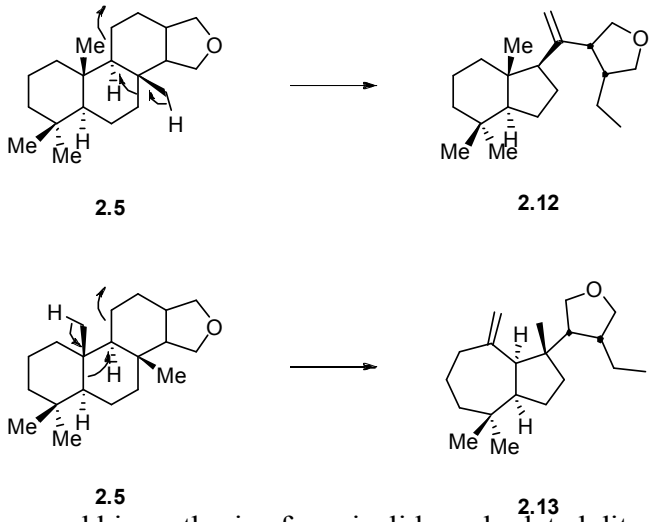
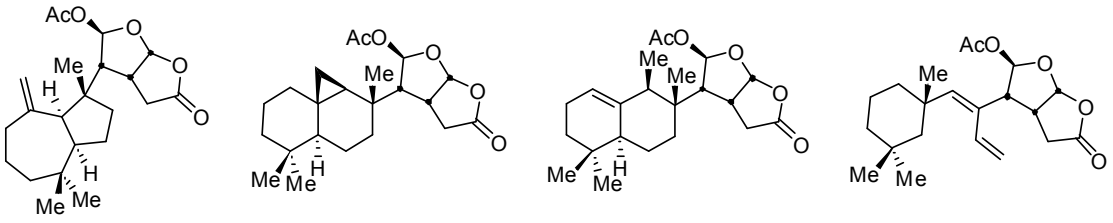


Figure 2.3 Proposed biosynthesis of norrisolide and related diterpene natural products.

The proposed biosynthesis of norrisolide from **2.5** (Figure 2.3) involves a ring contraction to form the 6-5 ring system followed by a ring opening that forms the upper ring system of the **2.12**.[1,9] It is also possible to access the core structure of the macfarlandins and the dendrillolides from **2.5** starting from a ring expansion of the 6-membered ring on the left, followed by a similar ring opening as above to access the 7-5 ring system with the exocyclic olefin in **2.13**.[10] Many of these compounds have been isolated together, giving more weight to the proposed biosynthesis.

There are many compounds that share the same oxygenated side chain as norrisolide. Dendrillolides A (**2.14**) and E (**2.15**) (Figure 2.4) were originally isolated from Dendrilla sp.;[11] however, structural reassignment occurred after subsequent

[9] It is likely that oxidation of unactivated **2.5** occurs prior to rearrangement to the carbon skeleton of norrisolide.
[10] Faulkner, D. J.; Sullivan, B. *J. Org. Chem.* **1984**, *49*, 3204-3206.
[11] Sullivan, B.; Faulkner, D. J. *J. Org. Chem.* **1984**, *49*, 3204-3206.



2.14 **2.15** **2.16** **2.17**

Figure 2.4 Natural products containing the norrisane side chain.

isolation from *Chromodoris macfarlandi* in 1989.[12] Also isolated from *C. macfarlandi* was macfarlandin C (**2.16**). While other members of the macfarlandin family, such as macfarlandins D and E have shown antimicrobial activity, macfarlandin C was inactive against all bacteria tested.[13] Spongionellin (**2.17**) was first reported in 1986 and was isolated from the sponge *Spongionella gracilis* in the Mediterranean Sea.

There are a variety of compounds that share norrisolide's 5-6 hydrindane ring system (Figure 2.5). Chelonoplysin (**2.18**), and the cheviolenes C (**2.19**) and E (**2.20**) were isolated from the marine sponge *Chelonaplysilla violacea*.[14] The hydrindane portions of all three molecules were identified by comparison to norrisolide. Norrlandin (**2.21**) was so named since it resembles a hybrid of norrisolide and macfarlandin E.

The chromodorolides A (**2.22**),[15] B (**2.23**)[16] and C (**2.24**)[17] can be found in the same aplysillid sponge. The chromodorolides have shown significant cytotoxicity

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[12] Bobzin, S. C Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 3902-2907.
[13] Molinki, T. F.; Faulkner, D. J.; Cun-Heng, H.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 4564-4567.
[14] Bergquist, P. R.; Bowden, B. F.; Cambie, R. C.; Craw, P. A.; Karuso, P.; Poiner, A. Taylor, W. C. *Aust. J. Chem.* **1993**, *46*, 623-632.
[15] Dumdei, E. J.; Dilip de Silva, E.; Andersen, R. J. *J. Am. Chem. Soc.* **1989**, *111*, 2712-2713.
[16] Morris, S. A.; Delip de Silva, E.; Andersen, R. J. *Can. J. Chem.* **1991**, *69*, 768-771.
[17] Rungprom, W.; Charvasiri, W.; Kokpol, U.; Kotze, A.; Garson, M. J. *Mar. Drugs* **2004**, *2*, 101-107.

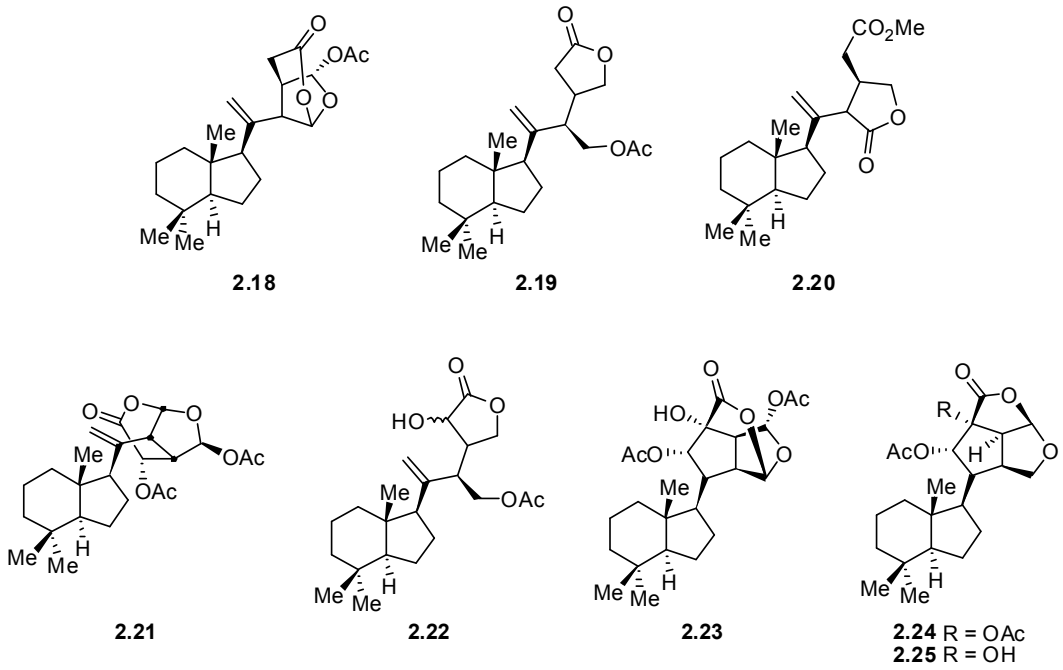


Figure 2.5 Natural products containing the hydrindane ring system.

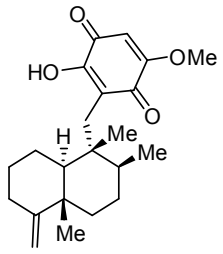
against the P388 mouse leukemia cell line. In addition, chromodorolide A has shown antimicrobial and nematocidal activity.[17] To the best of our knowledge other than norrisolide, it appears that no total synthesis of any of these molecules has been successfully completed.

Early biological activity reported for norrisolide includes secretary phospholipase A_2 ($SPLA_2$) inhibition,[18] ichthyotoxicity[19] and cyctotoxicitiy.[20] At a concentration of 2 μg/mL, norrisolide exhibits 66% inactivation of $SPLA_2$. Norrisolide was tested in a screen of compounds all containing a masked 1,4-dialdehyde moiety. Ichthyotoxicity was studied in a mosquito fish assay and was found to be toxic at 10 ppm. In this study,

[18] Potts, B. C. M.; Faulkner, D. J.. *J. Nat. Prod.* **1992**, *55*, 1701-1717.
[19] Gavagnin, M.; Vardaro, R. R.; Avila, C.; Cimino, G. *J. Nat. Prod.* **1992**, *55*, 368-371.
[20] Rudi, A.; Kashman, Y. *Tetrahedron* **1990**, *46*, 4019-4022.

norrisolide was found to be concentrated along the mantle (the outer edge) of the mollusc and also in the digestive tract. This finding helped strengthen the theory that norrisolide is a dietary metabolite.

Of particular interest to our lab was the biological activity first reported by Theodorakis in a report outlining a synthetic route to the norrisane side chain.[21] Theodorakis reported norrisolide irreversibly vesiculates the Golgi apparatus. Our previous studies on ilimaquinone (**2.26**) had our lab interested in molecules that vesiculate the Golgi.[22] Ilimaquinone reversibly vesiculates the Golgi by blocking new methylations in the cell. Addition of SAMe to the cell can reverse the effects of ilimaquinone. We were interested to learn if the biological target of norrisolide was related to that of ilimaquinone.

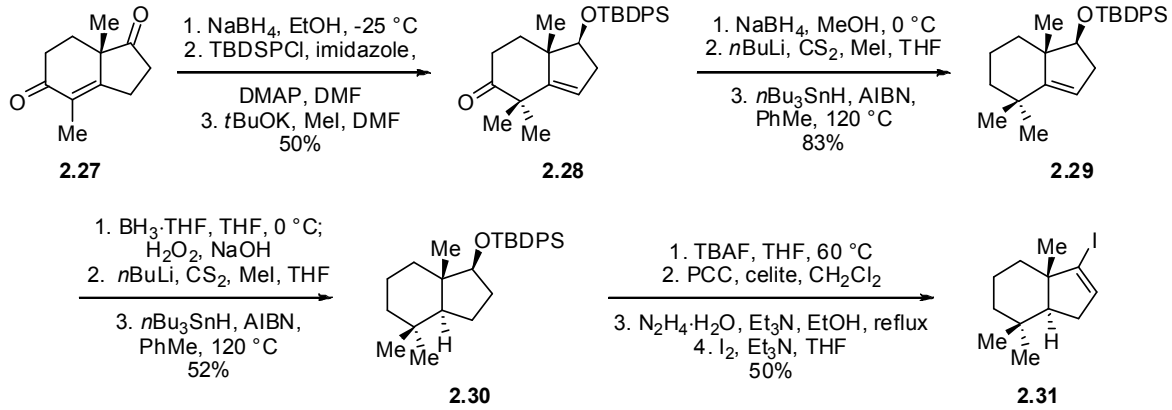


2.26

The Theodorakis group reported the first total synthesis of norrisolide in 2004.[23] The complete synthesis has a longest linear sequence of 28 steps from commercially available material. Starting from the Hajos-Parrish ketone **2.27**, it takes three steps to install the gem-dimethyl group in **2.28** (Scheme 2.1). Reduction and Barton-McCombie deoxygenation gives **2.29**, which in turn can undergo hydroboration to set the *trans* ring junction as a 2.5:1 (*trans*:*cis*) mixture of diastereomers. Another Barton-McCombie deoxygenation provides **2.30**. Four steps consisting of deprotection of the TBDPS group with TBAF, PCC oxidation, hydrazone formation and reaction with iodine under basic

[21] Kim, C.; Hoang, R.; Theodorakis, E. A. *Org. Lett.* **1999**, *1*, 1295-1297.
[22] Radeke, H. S.; Digits, C. A.; Casaubon. R. L.; Snapper. M. L. *Chem. Biol.* **1999**, *6*, 639-647.
[23] Brady, T. P.; Kim. S. H.; Wen, K.; Theodorakis, E. A. *Angew. Chem. Int. Ed.* **2004**, *43*, 739-742.

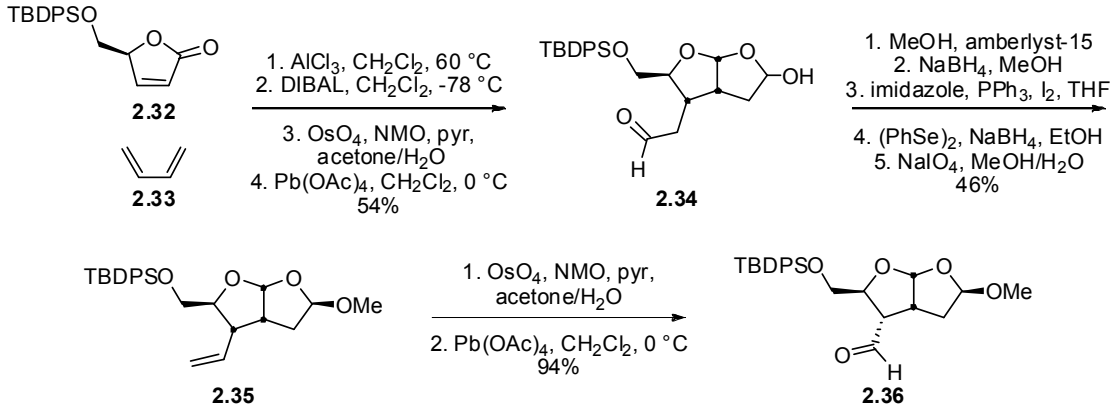
Scheme 2.1 Theodorakis' synthesis of a hydrindane intermediate.



conditions gives the vinyl iodide **2.31**, which is ready for coupling to the top half of the molecule.

As shown in Scheme 2.2, synthesis of the norrisane side chain began from the Diels-Alder cycloaddition of **2.32** and **2.33** under Lewis-acid mediated conditions to give the 5-6 system as a single isomer. DIBAL reduction of the lactone followed by oxidative cleavage of the olefin in the 6-membered ring gave **2.34**. The resulting lactol was protected as the methyl acetal and a series of six steps removed one carbon from the side

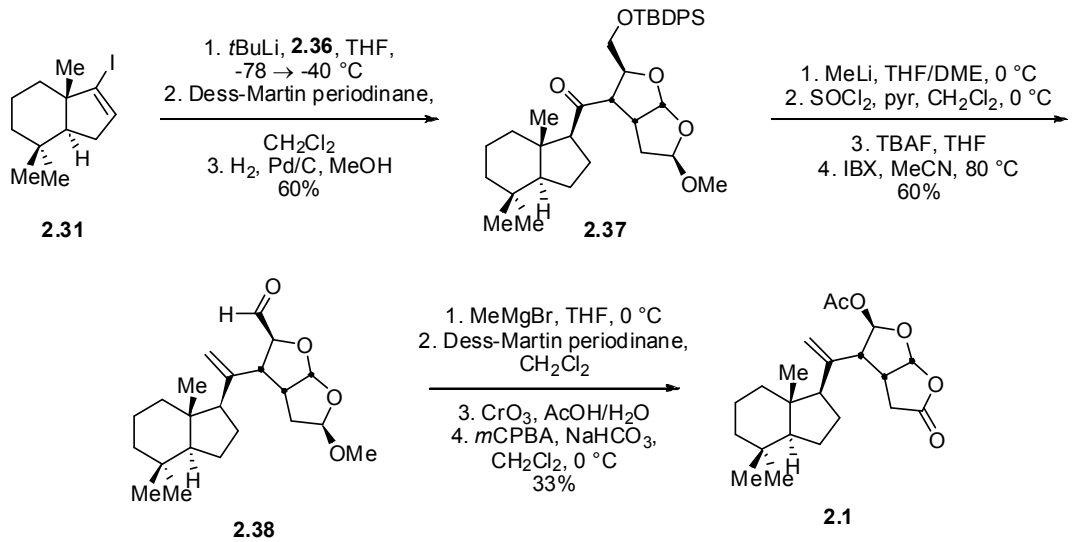
Scheme 2.2 Theodorakis' synthesis of the norrisane side chain.



chain to ultimately give **2.36** as the coupling partner for the top half of the molecule. This route improved upon a previous route from the same group that started from D-mannose.[21]

Assembly of the two coupling partners went smoothly (Scheme 2.3). Lithium-halogen exchange of vinyl iodide **2.31** followed by addition to aldehyde **2.36** gave the allylic alcohol, which was oxidized to the enone and the olefin reduced under standard conditions to give **2.37** as a single diastereomer. Methyl lithium addition followed by elimination gave the 1,1-disubstituted olefin in moderate yield. To finish the synthesis, the acetoxy group must be installed. This process began with silyl deprotection and oxidation of the resulting primary alcohol to the aldehyde (**2.38**). Methyl addition and re-oxidation gave the methyl ketone, which, after manipulation of the methyl acetal back to the lactone, was submitted to Bayer-Villager conditions to provide **2.1** in an overall yield

Scheme 2.3 The completion of Theodorakis' synthesis of norrisolide.



of 0.68%. It is important to note that at the beginning of our synthetic efforts these results were not yet published.

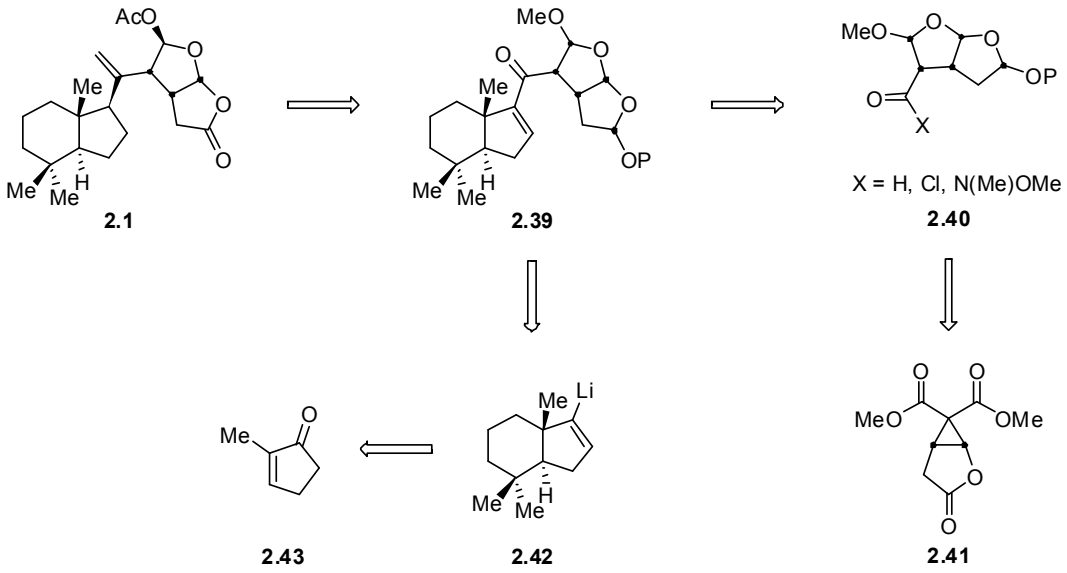
The Theodorakis group has also begun to investigate the biological activity[24] of norrisolide, although finding a suitable molecule for the identification of norrisolide's biological target has proven challenging.[25,26] Through testing various analogues it was shown that the hydrindane portion of the molecule is necessary to achieve biological activity; however, it is also necessary to have an electrophilic component in the upper portion of the molecule to achieve irreversible fragmentation of the Golgi complex. These results will be discussed in further detail in the next chapter.

2.2 Retrosynthetic Analysis

Our retrosynthetic plan starting from **2.1** brings us back through functional group manipulations and olefination to enone **2.39** (Scheme 2.4). This enone can be synthesized from the coupling of nucleophile **2.42** with electrophile **2.40**, which could take on the form of the aldehyde, acid halide or Weinreb amide. Electrophile **2.40** is accessed through the thermal rearrangement of cyclopropane **2.41**, which in turn can come from known compounds. Nucleophilic coupling partner **2.42** can be synthesized from the known ketone, which in turn can be brought back all the way to 2-methyl-2-

[24] Brady, T. P.; Wallace, E. K.; Kim, S. H.; Guizzunti, G.; Malhotra, V.; Theodorakis, E. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5035-5039. For a full report of the synthesis, see: Brady, T. P.; Kim, S. H.; Wen, K.; Kim, C.; Theodorakis, E. A. *Chem. Eur. J.* **2005**, *11*, 7175-7190.
[25] Guizzunti, G.; Brady, T. P.; Malhotra, V.; Theodorakis, E. A. *J. Am. Chem. Soc.* **2006**, *128*, 4190-4191.
[26] Guizzunti, G.; Brady, T. P.; Malhotra, V.; Theodorakis, E. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 320-325.

Scheme 2.4 Retrosynthetic analysis of norrisolide



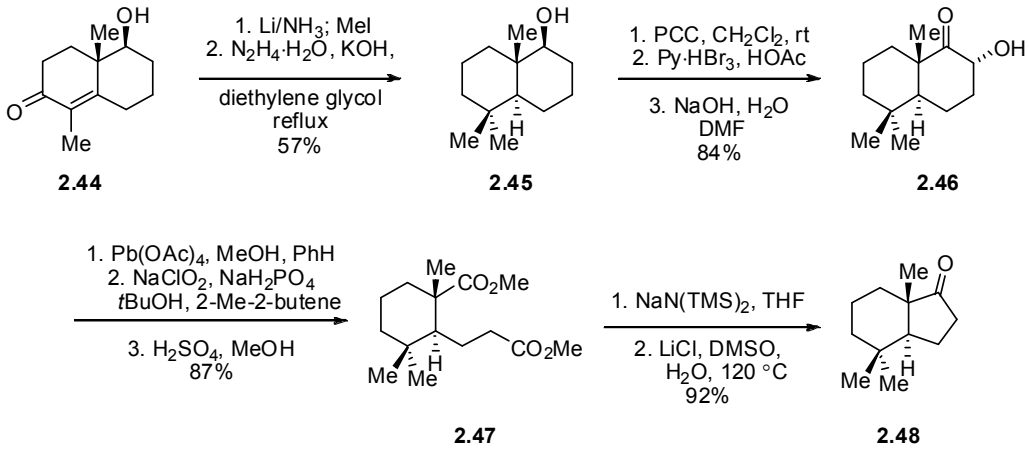
cyclopenten-1-one (**2.43**). This planned route generates a large degree of complexity with a minimum number of functional group manipulations.

2.3 Synthesis of the Hydrindane Portion

When our lab began synthetic efforts towards norrisolide, the state of the art for synthesizing the hydrindane portion of the molecule was a route developed by the Paquette group for their synthesis of *ent*-grindelic acid.[27] We were able to intersect this synthesis with intermediates known from our group's synthesis of ilimaquinone (Scheme 2.5).[28]

[27] Paquette, L. A.; Wang, H-L. *J. Org. Chem.* **1996**, *61*, 5352-5357.
[28] Bruner, S. D.; Radeke, H. S.; Tallarico, J. A.; Snapper, M. L. *J. Org. Chem.* **1995**, *60*, 1114-1115.

Scheme 2.5 Our first-generation synthesis of **2.48**.



Starting from **2.44**, lithium-ammonia reduction followed by trap with methyl iodide installed the gem-dimethyl compound **2.45**, which is necessary for the carbon framework of norrisolide. Wolff-Kishner reduction afforded **2.45**, which underwent PCC oxidation to the ketone. Bromination with pyridinium bromide perbromide followed by hydrolysis gave **2.46**. After a series of three steps, including ring opening through a lead tetraacetate cleavage, **2.46** is transformed to the dimethyl ester **2.47**. Dieckmann cyclization of **2.47** followed by decarboxylation gave **2.48** in a total of 10 steps from **2.44**. While this route was sufficient for the initial investigative stage of our synthesis, we strove to streamline the route to **2.48**, possibly through a ring-closing metathesis approach.

In that vein, work by the Lipshutz group outlining the conjugate addition of various allyl groups to cyclic enones[29] tied nicely into our synthetic plan. Taking the exact substrate from Lipshutz's report, enone **2.43** can be alkylated with the cuprate of

[29] Lipshutz, B. H.; Hackmann, C.; *J. Org. Chem.* **1994**, *59*, 7437-7444.

prenyl Grignard **2.49** at –78 °C (Scheme 2.6). We found that a modification of the procedure, including the use of HMPA as an additive,[30] helped to increase the yield and reproducibility of obtaining the TMS enol ether as opposed to the deprotected ketone (which was Lipshutz's ultimate desired product).

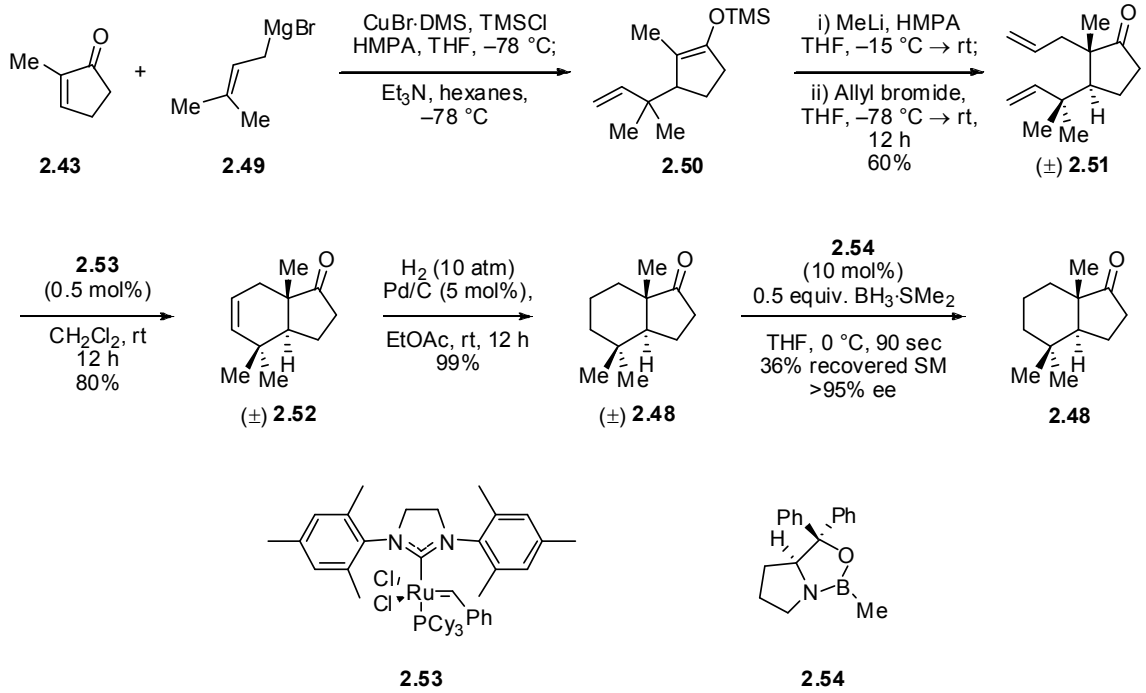
Attempts to allylate the protected enolate in one pot were unsuccessful; however, treatment of the unpurified TMS enol ether (**2.50**) with methyl lithium at –15 ˚C followed by cooling and addition of HMPA and allyl bromide[31] provided diene **2.51** in a 60% yield over two steps as a single diastereomer. This sequence can be carried out on a multi-gram scale. With the diene in hand, ring closing metathesis (RCM) with 0.5 mol% Grubbs' second generation catalyst (**2.53**) gives **2.52** in good yield, thus constructing the carbon framework for the hydrindane portion of the molecule in only three steps. Hydrogenation with palladium on carbon and slightly elevated hydrogen pressure (10 atm) cleanly gives (±) **2.48** quantitatively without the need for further purification.

Efforts to employ a one-pot tandem RCM/hydrogenation were unsuccessful without the addition of palladium on carbon before the hydrogenation took place. Also, purification of **2.52** directly after RCM is more straightforward than after being taken on crude through the hydrogenation. In addition, since (±)**2.48** does not require purification after simple hydrogenation, we felt that there was no large advantage to optimizing the tandem process.

---

[30] Williams, D. R.; Heidebrecht, R. W. Jr. *J. Am. Chem. Soc.* **2003**, *125*, 1843-1850.
[31] Cheung, A. K. Total syntheses of (+)- and (-)-Cacospongionolide B: investigation into structural requirements for phospholipase A2 inhibition. Ph.D. Dissertation, Boston College, Chestnut Hill, MA, 2003.

Scheme 2.6 Improved synthesis of ketone **2.48**.



To access optically pure **2.48,** it was necessary to perform a resolution. In lieu of a traditional resolution approach, we decided to carry out a kinetic resolution using CBS reduction. Using the proper antipode of the catalyst (**2.54**) should allow us to selectively reduce the undesired enantiomer of **2.48** while leaving the desired enantiomer at the ketone oxidation state. Indeed, this is the case in our reaction. Treatment of (±)-**2.48** with 10 mol% of (*S*)-(−)-2-methyl-CBS-oxazaborolidine (**2.54**) and 0.5 equivalents of borane-dimethylsulfide complex gives resolved **2.48** in 36% recovery. The enantiomeric excess of this reaction typically ranges from 95% to 98% ee, giving an *s* value in the range of 8.5 to 14.5, where an s value of 10 is generally considered synthetically useful.[32]

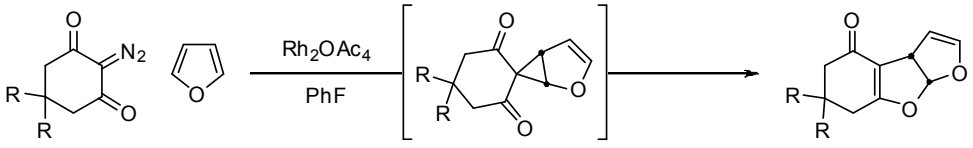
[32] Kagan, H. B.; Fiaud, J. C. *Top. Stereochem.* **1998**, *18*, 249-325.

The alcohol product can be reoxidized with PCC and reduced with the opposite antipode of the CBS catalyst to give the opposite enantiomer of **2.48** if desired.

2.4 Synthesis of the Norrisane Side Chain

While there are a handful of methods in the literature for synthesizing the tetrahydrofurofuranone ring system that is present in the upper portion of norrisolide, many were too lengthy or lacked the ability to easily introduce asymmetry.[33] Ultimately, it was decided that a formal 1,3-dipolar cycloaddition between a diester diazo compound and a furan derivative would be the most efficient way to access our carbon framework. Work done by the Pirrung group was the starting point for this investigation.[34] Pirrung showed that fused acetal products could be accessed in high yields starting from the cyclic diazo **2.55** (Scheme 2.7). Reaction of **2.55** with furan gives cyclopropane **2.57**, which spontaneously rearranges in *situ* to provide **2.58**.

Scheme 2.7 Pirrung's synthesis of fused acetal systems.

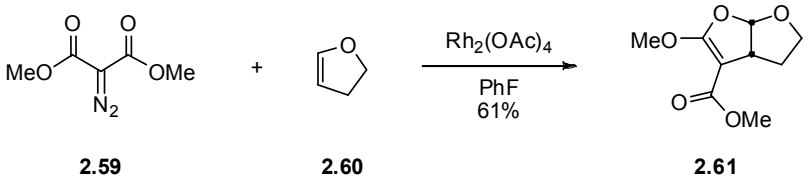


2.55 **2.56** **2.57** **2.58**

[33] For a more in-depth look at formation of these fused acetal systems, along with early studies on the norrisane side chain in our group, see: Casaubon, R. L. The Golgi-Disrupting Agents Ilimaquinone and Norrisolide: Determination of Biological Interactions Through Synthesis. Ph.D. Dissertation, Boston College, Chestnut Hill, MA, 2004.

[34] Pirrung, M. C.; Zhang, J.; Lackey, K.; Sternbach, D. D.; Brown, F. *J. Org. Chem.* **1995**, *60*, 2112-2124.

Scheme 2.8 Synthesis of the desired carbon framework of the fused acetal ring system.



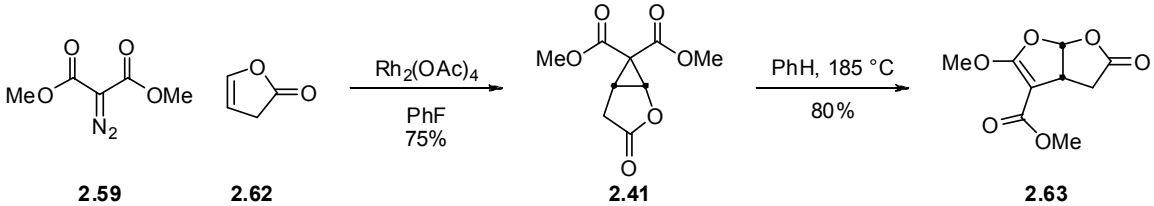
2.59 **2.60** **2.61**

An early attempt at this type of reaction in our group was between diazo **2.59** and dihydrofuran (**2.50**) using catalytic rhodium acetate, as shown in Scheme 2.8. This reaction demonstrated the fragmentation of the correct cyclopropyl bond, giving the desired product, as opposed to a ring opened olefinic compound.

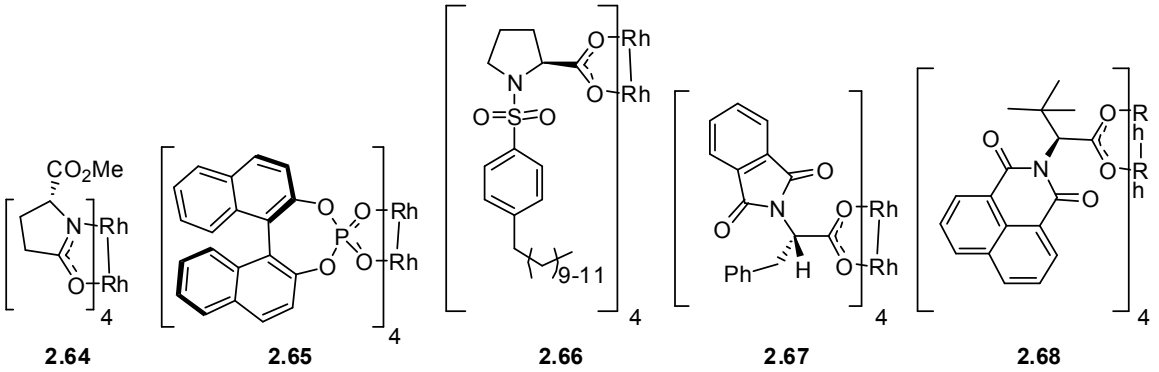
With the success of this test reaction, **2.60** was replaced with oxidized **2.62** (Scheme 2.9). What was obtained from the reaction of furanone **2.62** and diazo **2.59** was not, surprisingly, the fused acetal system. It was determined that the product of the reaction was cyclopropane **2.41**. Cyclopropanes had not previously been isolated in these types of reactions. However, it was postulated that the electron withdrawing nature of the lactone hindered the cleavage of the cyclopropane, therefore suppressing rearrangement.

Rearrangement of the isolated cyclopropane can be induced thermally under dilute conditions. Thermolysis of **2.41** in benzene [0.2 M] at 185 °C gives **2.63**, which does not require further purification. Using this method, we can synthesize the carbon skeleton of the norrisane side chain in just two steps.

Scheme 2.9 Cyclopropanation and rearrangement to access the fused acetal system.



2.59 **2.62** **2.41** **2.63**



2.64	2.65	2.66	2.67	2.68
10% yield	54% yield	52% yield	64% yield	60% yield
0% ee	42% ee	19% ee	34% ee	70% ee

Figure 2.6 Comparison of chiral catalysts for asymmetric cyclopropanation, showing yields and selectivities for **2.63**.

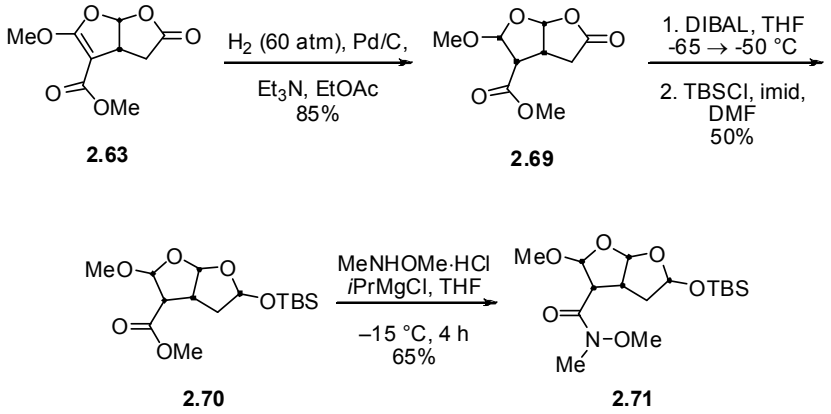
Access to an enantiomerically enriched norrisane side chain is necessary for our synthesis. Early attempts at using commercially available catalysts (Figure 2.6), such as Doyle's catalyst **2.64** and Davie's catalyst **2.66** gave low selectivities. The BINOL-derived catalyst **2.65** was more selective, giving 42% ee.

After testing various chiral Rhodium catalysts, our best results were with Müller's catalyst **2.68**.[35] We were able to obtain up to 70% ee on large scale by employing slow addition of the diazo compound to the reaction. The selectivity of this reaction is determined by chiral HPLC after thermolysis to **2.63**.

In addition to providing the best enantioselectivity for this reaction, catalyst **2.68** also increases the rate of the reaction from 12 hours (for the racemic reaction with $Rh_2(OAc)_4$) to two hours. This rate enhancement allows us to decrease the equivalents of **2.62**, which isomerizes into conjugation during the course of the reaction. With a faster

[35] Müller, P.; Allenbach, Y.; Robert, E. *Tetrahedron: Asymmetry* **2003**, *14*, 779-785.

Scheme 2.10 Elaboration of the norrisane side chain to Weinreb amide coupling partner.



reaction, and therefore shorter reaction times, the isomerization is less of a factor, thus allowing us to use only 2.5 equivalents of **2.62** as opposed to four in the racemic reaction.

Catalyst **2.68** is synthesized in two steps, beginning from *t*-leucine and 1,8-naphthalic anhydride. This adduct is then treated with rhodium acetate dimer with continuous removal of acetic acid through a Soxhlet extraction to provide **2.68** as a green solid that is air and moisture stable. After cyclopropanation, the catalyst can be precipitated with acetonitrile with 85% recovery and used one more time for cyclopropanation with the same efficiency.

As seen in Scheme 2.10, the synthesis continues with enantioenriched **2.63** being hydrogenated under 60 atm H_2 to provide **2.69**. This reaction must be buffered with one equivalent of triethylamine to counteract the inherent acidity of the palladium on carbon catalyst. This reaction occurs diastereoselectively to provide **2.69** with a *syn* relationship between all the hydrogens, introducing the cup-shaped ring system present in norrisolide.

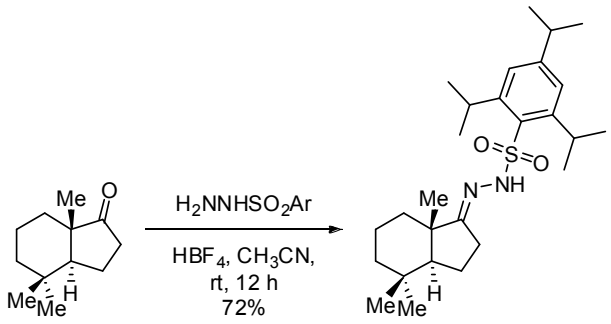
The lactone in **2.69** is reduced with DIBAL and transformed to the TBS acetal for protection from later chemistry, such as alkyl lithium and Grignard additions. The

reduction proceeds stereoselectively, with the hydride delivered solely from the top face of the molecule. The lactol is not isolated, but is taken directly on to the protection reaction, which affords clean **2.70** in 50% over the two steps. At this point, the methyl ester of **2.70** is transformed into the Weinreb amide, following the Merck procedure,[36] which is a suitable electrophile for fragment coupling. Early attempts in our group to synthesize the acid (and the subsequent acid chloride) or the aldehyde were not successful.

2.5 Fragment Coupling to Generate the Norrisolide Framework

To bring together the two ring system fragments, we decided to investigate a Shapiro coupling. To begin, we first needed to synthesize a suitable coupling partner from ketone **2.48** (Scheme 2.11). Reaction with 2,4,6-triisopropylphenylsulfonyl

Scheme 2.11 Transforming the hydrindane to a hydrazone for Shapiro coupling.



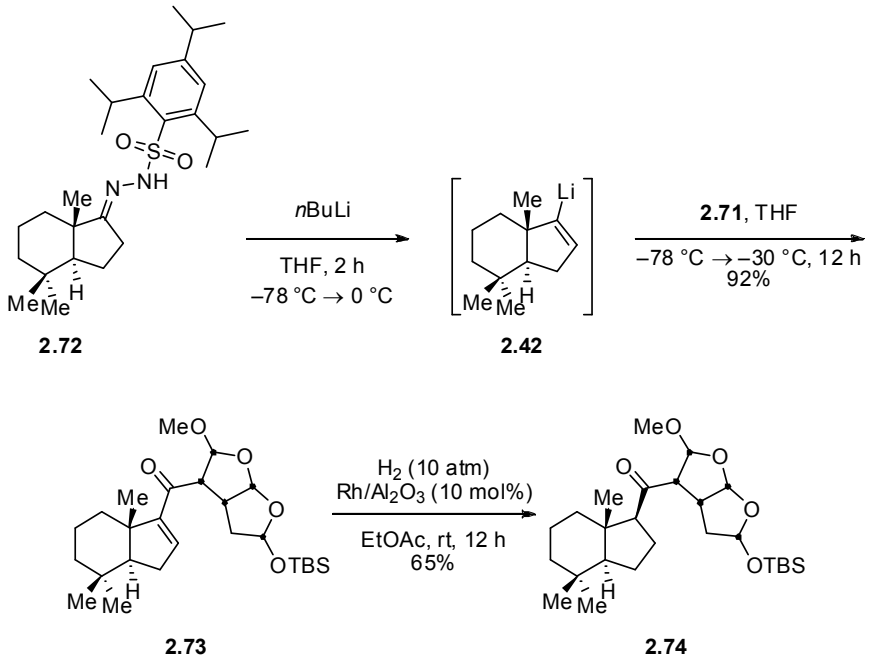
2.48 **2.72**

[36] Williams, J. M.; Jobson, R. B.; Yasuda, N.; Marchesini, G.; Dolling, U.-H.; Grabowski, E. J. J. *Tetrahedron Lett.* **1995**, *36*, 5461-5464.

hydrazide under acid catalysis gave **2.72** in 72% yield after recrystallization and drying over P_2O_5.

Treatment of **2.72** with two equivalents of *n*-BuLi gave the vinyl anion **2.42** in *situ*, which was in turn added to Weinreb amide **2.71**, giving enone **2.73** as a mixture of diastereomers stemming from the minor enantiomer from the cyclopropanation (Scheme 2.12). At this stage the diastereomers were not separable by chromatography and were taken on as a mixture through hydrogenation. Hydrogenation of **2.73** was found to work best with rhodium on alumina as a catalyst, using ethyl acetate as solvent. Methanol was also employed, but varying levels of water content occasionally caused epimerization α to the carbonyl. At this stage that the diastereomers resulting from the minor enantiomer of **2.71** were separated by silica gel chromatography.

Scheme 2.12 Coupling of the two fragments and diastereoselective hydrogenation.

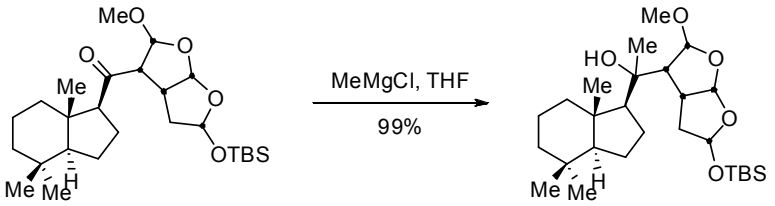


2.6 Olefination Studies

With **2.74** in hand, we needed to carry out the olefination of the bridging carbonyl. This process proved to be much more difficult than initially expected. Theodorakis' report was published just as we reached this step in our synthesis. As seen in Scheme 1, his team used a two-step approach to access the 1,1-disubstituted olefin, through addition of methyl Grignard and then elimination with thionyl chloride, since traditional olefination methods were not successful.

Efforts to use these conditions to olefinate **2.74** (Scheme 2.13) were not successful. Methyl Grignard added cleanly to the ketone, providing **2.75** in quantitative yield. However, attempts to eliminate water to form the 1,1-disubstituted olefin were unsuccessful. The complex mixture of products appeared to contain some tetrasubstituted olefin resulting from elimination toward the norrisane side chain. Attempts to optimize this process through base screens and other parameters such as temperature, time, concentration and other dehydrating agents[37] did not give satisfactory results.

Scheme 2.13 Addition of methyl Grignard in attempts at a two-step olefination procedure.



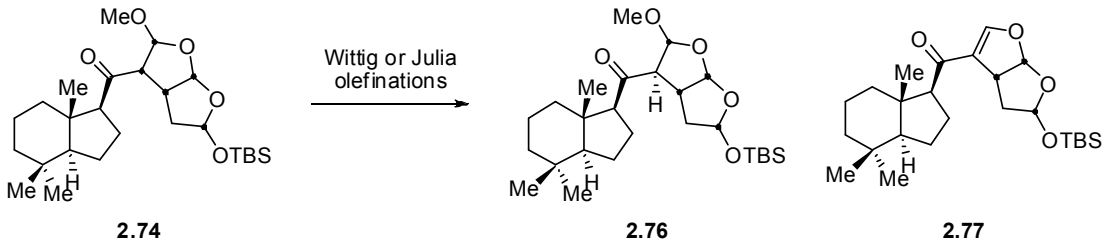
2.74 **2.75**

[37] Dehydration reagents designed to give the less substituted olefin, such as Martin sulfurane or Burgess reagent were unsuccessful, as these reagents afforded no reactivity even under forcing conditions.

Interestingly, studies on the opposite diastereomer (resulting from fragment coupling of the minor enantiomer of **2.71**) did produce the desired 1,1-disubstituted enone.[33] This seemingly unproductive pathway was, however, instrumental in being able to determine which enantiomer of cyclopropanation catalyst needed to be used to generate the correct cyclopropane enantiomer for the synthesis. The diastereomer may also be useful for future biological studies.

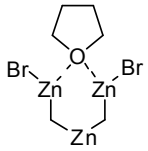
At this time, we decided to direct our attention to some one-pot olefination procedures. Theodorakis had reported that Wittig and Julia olefinations were not successful for them, and indeed we found this to be the case in our hands as well. Typical crude reaction mixtures from these olefinations generally contained a mixture of the epimerized ketone and elimination of the methoxy group, giving **2.76** and **2.77**, respectively, as shown in Scheme 2.14. Both of these products are hypothesized to arise from the harsh basic nature of these reagents. The carbonyl α proton in **2.74** is easily epimerized; simply leaving the compound at room temperature overnight results in minor amounts of the epimerized product.

Scheme 2.14 Typical results from Wittig or Julia olefinations of **2.74**.



2.74 **2.76** **2.77**

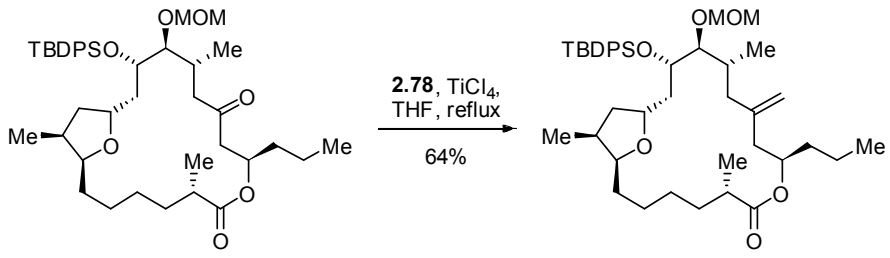
We felt $CH_2(MgX)_2$[38] was a promising reagent for a one-pot olefination, since methyl Grignard addition to **2.74** is a quantitative reaction. While the reagent could be prepared and used to olefinate benzophenone in yields similar to the original report, no reaction was obtained in the desired reaction. Using a similar concept of geminal dimetallic compounds, the reactive mixture of $Zn/CH_2Br_2/TiCl_4$ (the Takai olefination) has been used to rapidly and selectively olefinate compounds in studies on compounds in the gibberellin family.[39] This reagent has also been reported in large-scale reactions[40] and various methodologies.[41] Unfortunately, we again did not obtain any of the desired olefin.



2.78

Another geminal dimetallic compound that is popular in methenylation chemistry is the Nysted reagent (**2.78**).[42] The Nysted reagent has been used in Fürstner's synthesis of the amphidinolides (Scheme 2.15).[43] Ketone **2.79** turned out to be more sterically congested and less reactive than initially thought, and traditional olefination methods

Scheme 2.15 Fürstner's use of the Nysted reagent in olefination of an advanced intermediate in the synthesis of amphidinolides.



2.79 **2.80**

[38] Bertini, F.; Grasselli, P.; Zubiani, G. *Tetrahedron* **1970**, *26*, 1281-1290.
[39] Lombardo, L. *Tetrahedron Lett.* **1982**, *23*, 4293-4296.
[40] Lombardo, L. *Org. Syn.* **1987**, *65*, 81-85.
[41] a) Takai, K.; Kakiuchi, T.; Kataoka, Y.; Utimoto, K. *J. Org. Chem.* **1994**, *59*, 2668-2670. b) Matsubara, S.; Mizuno, T.; Otake, T.; Kobata, M.; Utimoto, K.; Takai, K. *Synlett*, **1998**, 1369-1371.
[42] Matsubara, S.; Sugihara, M.; Utimoto, K. *Synlett*, **1998**, 313-315.

were ineffective. The researchers sought a method that would allow the reaction to take place at elevated temperature to increase the flexibility and conformation of the macrocycle to allow for the ketone center to become more sterically available. Also, a method that avoided base was desired to avoid opening of the macrolactone. The Takai olefination was successful for this reaction; however, the results were not consistent. Instead, the Nysted reagent with $TiCl_4$ as a promoter gave 1,1-disubstited olefin **2.80** in 64% yield. Unfortunately, even given this precedent, the Nysted reagent did not affect the olefination of **2.74**, resulting in no reaction even in refluxing THF.

In 2004, the Lebel group published two reports outlining a rhodium-catalyzed methenylation using TMS diazomethane, of both aldehydes and ketones.[44,45] This reaction does not require addition of base and is reported to be mild enough for sensitive and enolizable substrates. Unfortunately, to olefinate aliphatic ketones, this reaction requires elevated temperatures, which ultimately epimerized the ketone, giving **2.76**. Similar results were obtained with the Petasis reagent (Cp_2TiMe_2), which also does not require the addition of base but does require elevated temperatures to generate the reactive species.

Investigations into the Peterson olefination[46] started with addition of $TMSCH_2MgCl$ into the ketone **2.74** to give **2.81** in quantitative yields (Scheme 2.16). The more widely used, and commercially available, $TMSCH_2Li$ was not successful in

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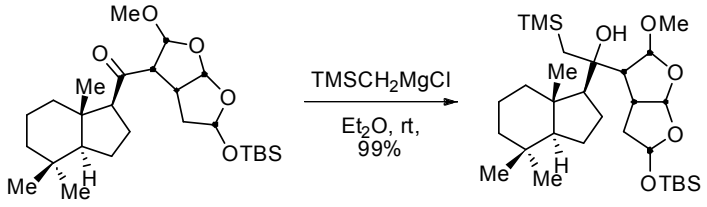
[43] Aïssa, C.; Fiveiros, R.; Ragot, J.; Fürstner, A. *J. Am. Chem. Soc.* **2003**, *125*, 15512-15520.
[44] Lebel, H.; Guay, D.; Paquet, V.; Huard, K. *Org. Lett.* **2004**, *6*, 3047-3050.
[45] Lebel, H.; Paquet, V. *J. Am. Chem. Soc.* **2004**, *126*, 320-328.
[46] a) Hudrlik, P. F.; Peterson, D. *Tetrahedron Lett.* **1972**, 1785-1787. b) For a more in-depth discussion of this reaction, see: van Staden, L. F.; Gravestock, D.; Ager, D. J. *Chem. Soc. Rev.* **2002**, *31*, 195-200.

Scheme 2.16 Addition of $TMSCH_2MgCl$ to **2.74** as the first step in the Peterson olefination.



2.74 **2.81**

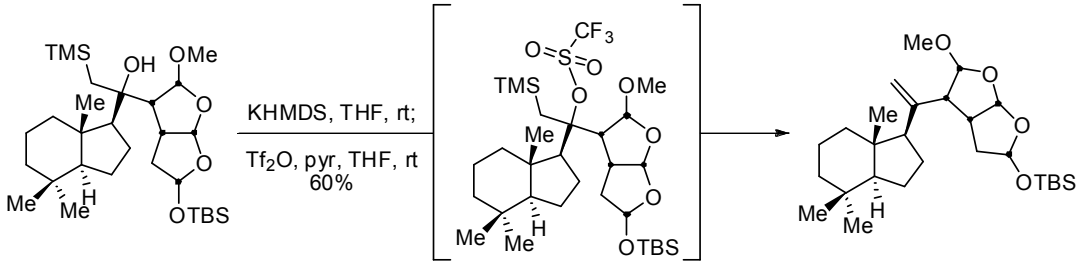
this reaction, even using the common and often necessary additive $CeCl_3$.[47] These conditions resulted in decomposition of the starting material without producing any desired product. Preparing the same reagent fresh did not have any effect on the outcome of the reaction.

Attempts at elimination of **2.81** began with one of the most general procedures, simple treatment with KH in ethereal solvents, such as THF, diethyl ether and dioxane. These conditions did give trace amounts of desired product during one early attempt; however, these results were not reproducible. Additives such as HMPA, TMEDA or crown ethers did not have any favorable influence on the reaction, nor did elevating the temperature. Switching to NaH or KHMDS, other common bases for this reaction, also did not provide the desired product.

Acidic conditions, from an early report by Peterson,[46] using acetic acid saturated with sodium acetate only afforded deprotection of the TBS group. Even continuing to push the reaction after this deprotection did not lead to any of the 1,1-disubstituted olefin. A review on methylenations[48] brought to our attention an alternative elimination method

[47] Johnson, C. R.; Tait, B. D. *J. Org. Chem.* **1987**, *52*, 281-283.
[48] Beadham, I.; Micklefield, J. *Curr. Org. Syn*. **2005**, *2*, 231-259.

Scheme 2.17 Elimination to the 1,1-disubstituted olefin using triflic anhydride.



2.81 **2.82** **2.83**

to complete the Peterson olefination transformation. Treatment of the alkoxide, after addition of the Grignard or lithium reagent, with thionyl or acetyl chloride achieved the formation of methylenecyclohexene in quantitative yields from cyclohexanone, whereas acidic conditions isomerize the 1,1-disubsituted olefin into the ring.[49]

Attempting these conditions, using acetyl chloride as a quench for the addition of $TMSCH_2MgCl$ did not produce any olefinic product, only **2.81**. This reaction was less clean than the standard reaction. Independent deprotonation of **2.81** with KHMDS and subsequent treatment with AcCl produced a complex mixture of products, which contained a minor amount of the desired olefin. Adding triethylamine or pyridine did not increase the amount of product produced, nor did it clean up the reaction mixture. Thionyl chloride in the place of acetyl chloride did not produce any desired product. However, replacing the acetyl chloride with triflic anhydride and using excess pyridine cleanly gave the 1,1-disubsituted olefin **2.83** as the only product (Scheme 2.17).

With **2.83** in hand, we were able to continue in the synthesis, with the remaining steps all occurring on the periphery of the molecule, consisting of deprotection and

[49] Chan, T. H.; Chang, E. *J. Org. Chem.* **1974**, *39*, 3264-3268.

oxidation back to the lactone and manipulation of the methyl acetal by epimerization and acetylation.

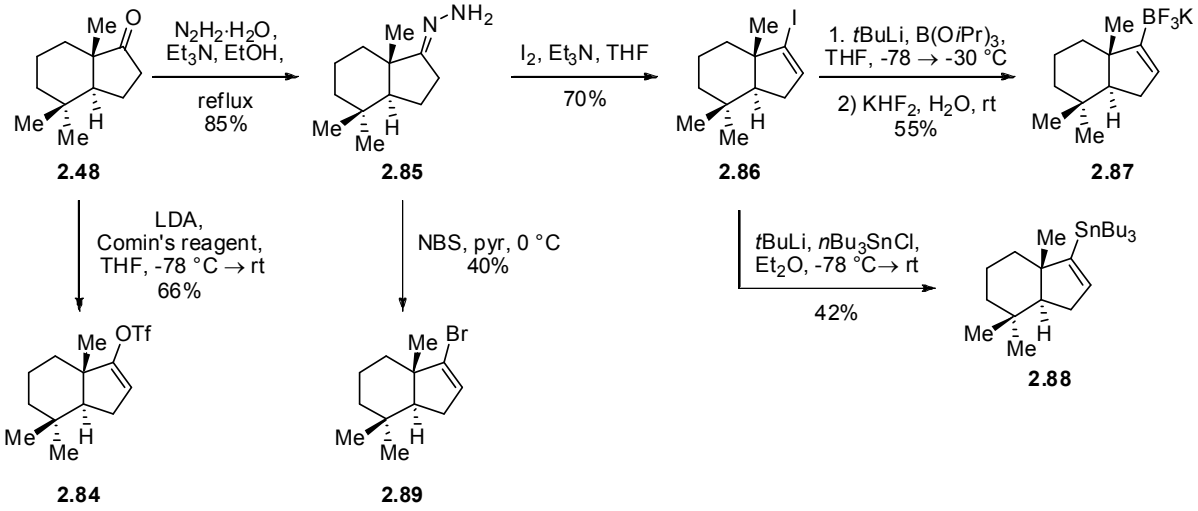
2.7 Studies on an Alternative Route to Install the 1,1-Disubstititued Olefin

In addition to olefination, we also looked into installing the 1,1-disubstituted olefin before coupling the two fragments through a metal-catalyzed reaction. We were able to make a variety of coupling partners, such as vinyl halides, triflates, stannanes and borates, as shown in Schemes 2.18 and 2.19.

Ketone **2.48** can be elaborated to the vinyl triflate **2.84** through treatment with LDA and then Comins reagent (*N*-(5-chloro-2-pyridyl)triflimide)[50] in 66% yield. **2.48** can also be converted to vinyl halides through the hydrazone **2.85**. Treatment with elemental iodine or NBS gives vinyl iodide **2.86** and vinyl bromide **2.89**, respectively. The vinyl iodide was further transformed to the stannane **2.88** through lithium-halogen exchange followed by trap with tributyltin chloride, giving a 2:1 mixture of desired product to proton-quenched product. The undesired product is simply removed by high vacuum, leaving the desired vinyl stannane behind in 42% yield. Another cross-coupling partner, the vinyl trifluoroborate, can also be generated in 55% yield from **2.86** through lithium-halogen exchange followed by trapping with potassium bifluoride and water. **2.87** is not stable enough for long-term storage, so it must be prepared fresh for each use.

[50] Comins, D. L.; Dehghai, A. *Tetrahedron Lett.* **1992**, *33*, 6299-6302.

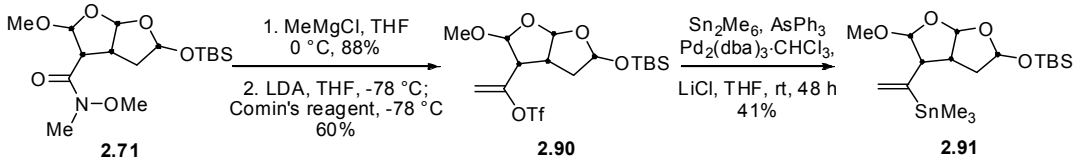
Scheme 2.18 Synthesis of coupling partners of the hydrindane portion of the molecule.



To generate coupling partners for the norrisane side chain, we must begin by adding one carbon. From **2.71**, methyl Grignard is added in 88% yield. This can then be elaborated to the vinyl triflate in conditions similar to above. The triflate (**2.90**) can also be further converted to the vinyl stannane through Stille cross coupling conditions with hexamethylditin in 41% yield.

Unfortunately, all the cross coupling reactions tried between the hydrindane and norrisane portions were unsuccessful. Negishi, Suzuki, and Stille couplings were all attempted, and all gave no reaction. We were able to couple test reagents, such as phenyl trifluoroborate to **2.90**, but no test couplings between the hydrindane coupling partners

Scheme 2.19 Synthesis of the coupling partners of the norrisane portion of the molecule.

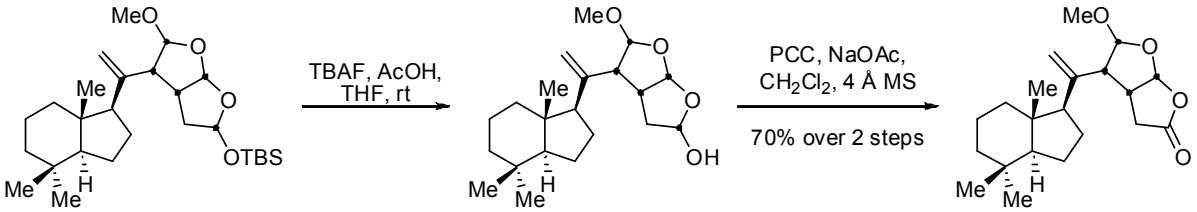


2.84, 2.86, 2.87, 2.88 or **2.89** were successful under thermal or microwave conditions. We have hypothesized that the ring junction methyl group imposes an intense steric hindrance to the cross coupling reaction. However, at this time our olefination studies were improving, so it was determined that studies on this route were no longer necessary.

2.8 Completion of the Synthesis

To complete our synthesis, once the 1,1-disubstituted olefin was installed, only three transformations remained. To begin, we deprotected the TBS acetal of **2.83** (Scheme 2.20) with TBAF buffered with acetic acid. This lactol was not purified, but oxidized directly with PCC. This gave the lactone **2.92** in 70% yield over two steps, leaving one final transformation. In earlier studies[33] and in model studies, it was found that a mixture of acetic and trifluoroacetic acids could epimerize and install the acetate

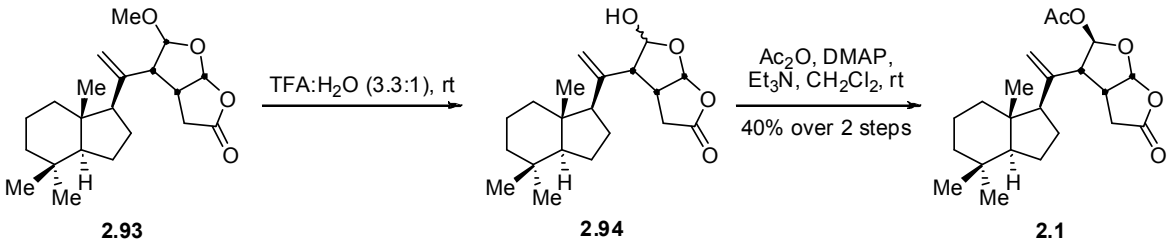
Scheme 2.20 Deprotection and oxidation of the norrisane side chain.



2.83 **2.92** **2.93**

group necessary for the natural product. Unfortunately, these conditions provided a mixture of epimers, the minor of which was determined to be norrisolide.

Scheme 2.21 Completion of the total synthesis of norrisolide.



2.93 **2.94** **2.1**

We were able to complete this transformation by first hydrolyzing the methyl acetal to the lactol with a 3.3:1 mixture of trifluoroacetic acid to water gave a 1:1 mixture of lactol epimers (**2.94**) (Scheme 2.21). Upon protection as the acetate using standard conditions gave only one compound in 40% yield over the two steps. We identified this compound as **2.1**, which matched spectral data with that of natural norrisolide.

2.9 Conclusion

We have completed the total synthesis of norrisolide in a longest linear sequence of 13 steps, with an overall yield of 1.7%. Our route improves on the previously published route to this molecule, using less than half the number of steps and doubling the overall yield. This synthesis employs a rapid entry into the hydrindane core, along with access to the norrisane side chain without having to rely on the chiral pool.

2.10 Experimental and Supporting Information

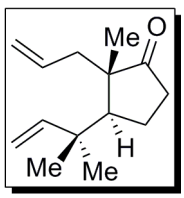
General Methods

Reagents were purchased from commercial suppliers and used without further purification, with the exception of the following: HMPA, TMSCl, Et_3N, allyl bromide, acetic anhydride and ethyl acetate were distilled over CaH_2. Tetrahydrofuran, diethyl ether, dichloromethane and benzene were dried on alumina columns using a solvent dispensing system. Hexanes was distilled prior to being used as a chromatography solvent.

Reactions were run in oven dried (140 °C, overnight) glassware under an inert atmosphere of dry nitrogen unless otherwise noted. Liquids were transferred to the reaction flask via syringe through rubber septa. All reactions were stirred with PTFE covered stirbars. Air and moisture sensitive compounds were transferred in a glove box under nitrogen atmosphere. Concentration refers to the removal of solvent using a Büchi rotary evaporator at 40 torr followed by a vacuum pump at 1 torr. Base washed silica gel was prepared by washing with 1% Et_3N in EtOAc or Et_2O (depending on the eluent mixture of the column) followed by 100% EtOAc or Et_2O and then equilibrating with the solvent mixture to be used for elution. Cerium ammonium molybdate was used as the primary thin layer chromatography (TLC) staining reagent. Silica gel column chromatography was performed using Baxter brand silica gel (60 Å, 230-240 mesh ASTM.)

^{1}H NMR spectra were commonly recorded on a Varian GN-400 (400 MHz) spectrometer, but in some cases a Varian Unity 300 (300 MHz) was used. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CHCl_3$, δ 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constant (Hz), and integration. ^{13}C NMR were recorded on a Varian GN-400 (100 MHz) or a Varian Gemini-500 (125 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CHCl_3$, δ 77.2). Infrared (IR) spectra were obtained using Nicolet Avatar 360 Spectrophotometer, $\bar{\nu}_{max}$ in cm^{-1}. High-resolution mass spectral analyses (HRMS) were performed using ESI^+ or $DART^+$ as the ionization technique. Enantiomeric ratios were determined by chiral HPLC on a Shimadzu chromatograph with a Chiralcel OD column (4.6 x 250 nm) by Chiral Technologies (90:10 hexanes:isopropanol, detected at 230 nm) or by Chiral GC analysis Supelco Betadex 120 column (30 m x 0.25 mm). Optical rotations were measured on a Rudolph Research Analytical Autopol IV polarimeter.

Synthetic Procedures

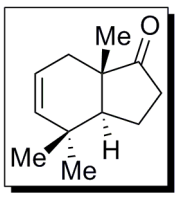


Diene 2.51: To a suspension of CuBr·DMS (16.28 g, 79.2 mmol) in THF (150 mL) at –78 ºC was added a solution of prenyl Grignard (440 mL, 0.15 M in THF, 66.0 mmol) dropwise via cannula. The reaction was allowed to stir at –78 ºC for 15 min. HMPA (18.4 mL, 106 mmol) was then added, and the reaction was allowed to stir for an additional 20 min at –78 ºC. A solution of 2-

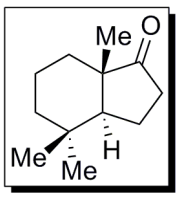
methyl-2-cylopenten-1-one (5.2 mL, 52.8 mmol), TMSCl (13.4 mL, 106 mmol) and THF (25 mL) was then added over 2 h via syringe pump, and the reaction mixture was allowed to stir for an additional hour. A solution of Et_3N (16.2 mL, 116 mmol) in hexanes (60 mL) was then added over 1 h via syringe pump. The reaction was allowed to stir 1 h at -78 ºC and was then warmed to 0 ºC for 30 min. The reaction was diluted with Et_2O (400 mL) and added to a cold 10% saturated solution of NH_4Cl (600 mL) in a separatory funnel. The layers were separated, and the organic layer was washed with portions of cold 10% saturated aqueous solution of NH_4Cl (300 mL each) until the aqueous layer no longer turned blue. The organic layer was then washed with brine, dried with $MgSO_4$ and concentrated. The crude TMS enol ether (a yellow oil) was taken on immediately without purification.

To a solution of the enol ether (52.8 mmol) in THF (240 mL) at –15 ºC was added methyl lithium (38.5 mL, 1.37 M in Et_2O, 52.8 mmol). The reaction was allowed to stir and warm to room temperature over 1 h. The reaction mixture was then cooled to –78 ºC and HMPA (36.8 mL, 211 mmol) was added. After 15 min allyl bromide (23.0 mL, 264 mmol) was added. The reaction mixture was allowed to stir and warm to room temperature over 16 h. The reaction was then treated with 1 M HCl (300 mL), allowed to stir for 5 min, and then diluted with Et_2O (200 mL). The layers were separated and the aqueous layer was extracted with Et_2O (3 x 100 mL), dried with $MgSO_4$, and concentrated. The crude yellow oil was purified by silica gel chromatography (100% hexanes to 50:1 hexanes:Et_2O) to provide the diene as a clear and colorless oil (6.8 g, 62%). $\mathbf{R_f}$ =0.26 (10:1 hexanes:Et_2O); **IR** (thin film) $\bar{\nu}_{max}$ 3078, 2969, 2362, 2340, 1740,

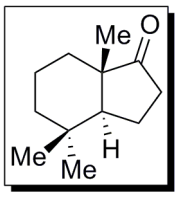
1638, 1460, 1107, 998, 904 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.98 (dd, *J* = 17.6, 10.4 Hz, 1H), 5.51 (dddd, *J* = 14.8, 10.4, 8.8, 6.0 Hz, 1H), 5.06-4.95 (m, 4 H), 2.50 (ddt, *J* = 14.4, 6.0, 1.6 Hz, 1H), 2.40-2.27 (m, 2H), 2.08-1.91 (m, 3H), 1.76-1.68 (m, 1H), 1.15 (s, 3H), 1.11 (s, 3H), 0.95 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 223.2, 147.4, 135.1, 118.3, 111.0, 53.6, 50.2, 42.5, 39.7, 37.9, 27.8, 26.3, 21.3, 20.3; **HRMS**-DART (*m/z*) calcd for $C_{14}H_{23}O$ [M^+] 207.1749, found 207.1755.



Ketone 2.52: To diene **2.51** (5.47 g, 26.5 mmol) dissolved in CH_2Cl_2 (132 mL) was added Grubbs' second-generation catalyst (0.11 g, 0.13 mmol). The reaction was allowed to stir for 16 h open to air. At the completion of the reaction silica gel (5 g) was added and the reaction was allowed to stir for an additional 30 min. The reaction was then filtered and concentrated. The resulting yellow oil was purified by silica gel chromatography (50:1 to 20:1 pentane:Et_2O) to provide a volatile, pale yellow oil (3.9 g, 83%). $\mathbf{R}_f$ = 0.56 (5:1 hexanes: Et_2O); **IR** (thin film) $\bar{\nu}_{max}$ 2957, 2913, 1737, 1464, 1065, 1029, 723 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.53 (ddd, *J* = 10.0, 4.8, 2.8 Hz, 1H), 5.45 (dt, *J* = 10.0, 1.6 Hz, 1H), 2.46 (ddd, *J* = 19.2, 8.4, 0.8 Hz, 1H), 2.13 (dd, *J* = 19.2, 9.6 Hz, 1H), 2.02 – 1.95 (m, 3H), 1.81 (dddd, *J* = 12.0, 11.4, 10.4, 9.4 Hz, 1H), 1.65 (dd, *J* = 13.2, 5.6 Hz, 1H), 1.04 (s, 3H), 1.03 (s, 3H), 0.98 (s, 3H); **^{13}C NMR** (100 MHz, CDCl3, δ) 221.9, 138.2, 122.1, 51.4, 47.1, 36.1, 35.8, 33.7, 31.7, 23.1, 19.8, 16.7; **HRMS**-DART (*m/z*) calcd for $C_{12}H_{19}O_1$ [M^+] 179.1436, found 179.1431.



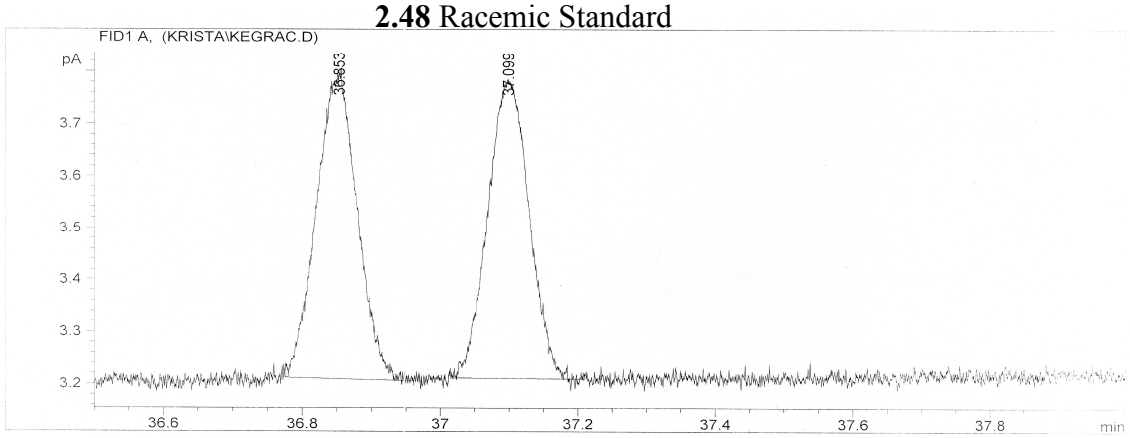
Hydrindane (±)-2.48: To a suspension of Pd/C (0.67 g, 10 wt%, 0.59 mmol) in EtOAc (20 mL) in a bomb sleeve was added ketone **2.52** (2.1 g, 11.8 mmol) in EtOAc (40 mL). The reaction mixture was placed in a Parr bomb. The bomb was sealed, flushed with H_2 and then pressurized to 10 atm H_2. After 16 h with vigorous stirring the reaction mixture was filtered through celite with EtOAc (80 mL) to give the desired compound as a clear, colorless, volatile oil (2.05 g, 96%). $\mathbf{R}_f$ = 0.26 (10:1 hexanes:Et_2O); **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 2.42 (ddd, *J* = 19.2, 9.2, 1.2 Hz, 1H), 2.06 (dt, *J* = 18.0, 8.8 Hz, 1H), 1.88 (dddd, *J* = 12.0, 9.2, 5.6, 0.8 Hz, 1H), 1.8-1.4 (m, 6H), 1.29 (dd, *J* = 13.2, 5.6 Hz, 1H), 1.14 (m, 1H), 0.94 (s, 6H), 0.87 (s, 3H).



(+)-Hydrindane 2.48: To a solution of (*S*)-(−)-2-methyl-CBS-oxazaborolidine (0.55 mL, 1 M PhMe, 0.56 mmol) in THF (27 mL) in a 1 L round bottom flask at 0 ºC was added $BH_3{\cdot}SMe_2$ (0.26 mL, 2.78 mmol). The reaction mixture was allowed to stir at 0 ºC for 15 min, at which time ketone **(±)-2.48** (1.0 g, 5.5 mmol) was added in THF (27 mL). The reaction mixture was allowed to stir for 2 min at 0 ºC, then MeOH (20 mL) and 1 M HCl (20 mL) were added and the reaction mixture was warmed to room temperature and allowed to stir for 1 h. Et_2O (100 mL) was added and the layers separated. The aqueous layer was extracted with Et_2O (3 x 20 mL), and the combined organic layers were washed with brine (2 x 40 mL), dried with $MgSO_4$ and concentrated to give a crude white oil. The crude compound was purified by silica gel chromatography (20:1 pentanes:Et_2O elute the ketone, then 1:1 hexanes:Et_2O to elute

the alcohol). The ketone was provided as a clear, colorless, volatile oil (0.32 g, 32%).$\mathbf{R}_f$ = 0.26 (10:1 hexanes:Et_2O); $[\alpha]_D^{20}$ = +49.9 (*c* 3.8, CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 2.42 (ddd, *J* = 19.2, 9.2, 1.2 Hz, 1H), 2.06 (ddd, *J* = 18.0, 8.8, 8.8 Hz, 1H), 1.88 (dddd, *J* = 12.0, 9.2, 5.6, 0.8 Hz, 1H), 1.8-1.4 (m, 6H), 1.29 (dd, *J* = 13.2, 5.6 Hz, 1H), 1.14 (m, 1H), 0.94 (s, 6H), 0.87 (s, 3H).

2.48 Racemic Standard



Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
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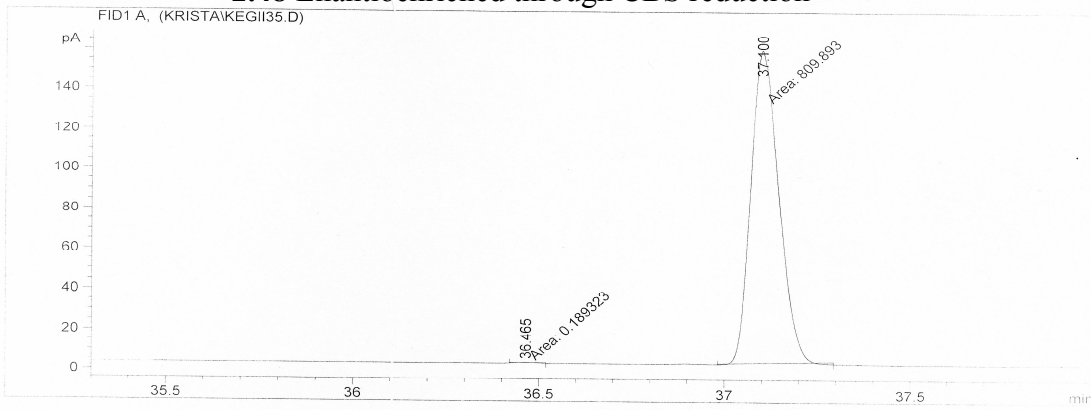
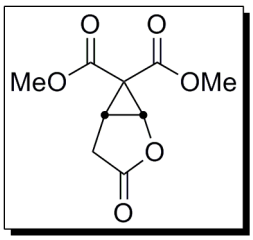
2	37.099	1	PB	2.40326	5.69346e-1	49.78720

2.48 Enantioenriched through CBS reduction

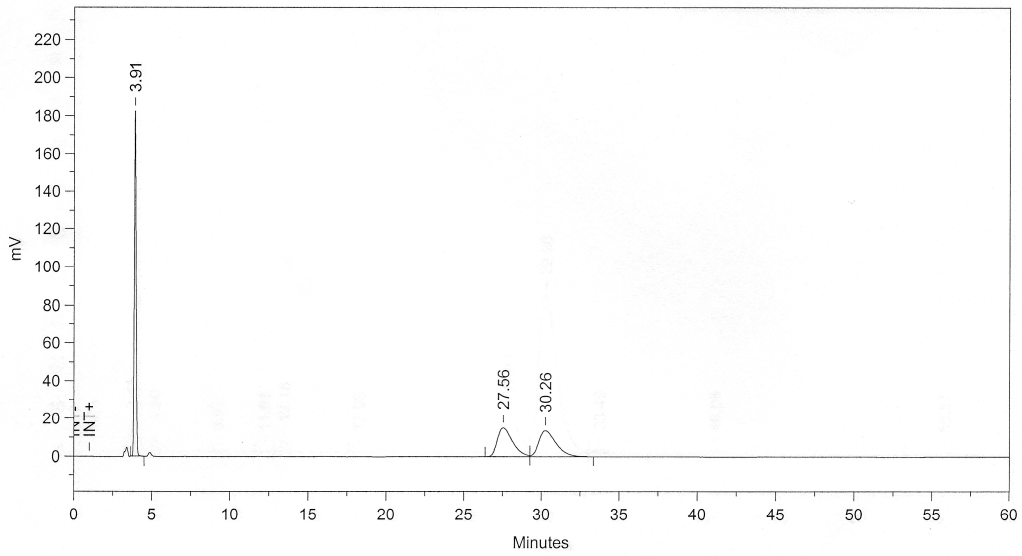


Peak #	RetTime [min]	Sig	Type	Area [pA*s]	Height [pA]	Area %
1	36.465	1	MM	1.89323e-1	1.23469e-1	0.02337
2	37.100	1	MM	809.89319	155.94565	99.97663
Totals :				810.08251	156.06912	



Cyclopropane 2.41: To rhodium catalyst **2.68** (0.86 g, 0.59 mmol) was added PhF (30 mL) and furan-2(3H)-one (5.2 mL, 74 mmol). A solution of dimethyl 2-diazomalonate (4.0 mL, 29 mmol) in PhF (20 mL) was added to the reaction mixture over 2 h via syringe pump. The reaction mixture was allowed to stir for an additional hour after the addition, at which time the reaction was concentrated. The resulting thick green oil was added dropwise to CH_3CN (400 mL), where the catalyst precipitated as a purple solid. This was filtered and concentrated. The crude light green oil was purified by base washed silica gel chromatography (2:1 hexanes:Et_2O) to give the product as a yellow oil (4.36 g, 70%). $\mathbf{R_f}$ = 0.43 (1:1 hexanes:EtOAc); $\mathbf{[\alpha]_D^{20}}$ = -51.5 (*c* 1.60, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 3073, 3008, 2870, 2851, 1589, 1469, 1423, 1042, 722, 698, 598 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 4.78 (d, *J* = 6.4 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.08 (dd, *J* = 19.0, 0.8 Hz, 1H), 2.92 (dd, *J* = 19,0, 6.8 Hz, 1H), 2.54 (ddd, *J* = 7.2, 6.4, 0.8 Hz, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$, δ) 175.0, 167.1, 164.4, 63.5, 53.4, 37.5, 29.7, 25.8; **HRMS-ESI** (*m/z*) calcd for $C_9H_{11}O_6$ [M^+] 215.0556, found 215.0560.

2.41 Racemic Standard



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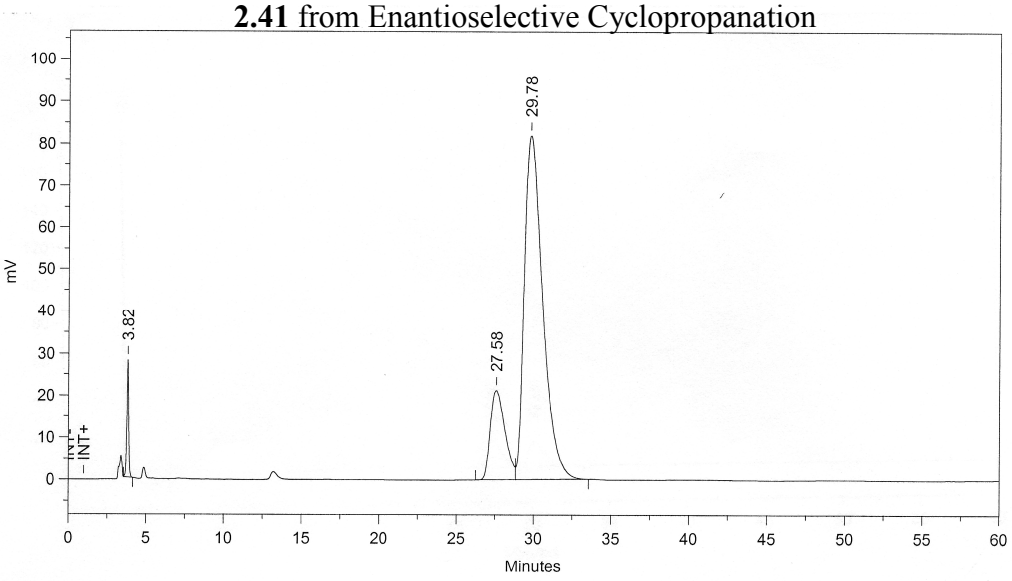
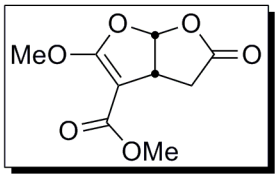
3	30.259		0.0000	0.000	1030387.0	29.120	VB	1.277	13449.48	6.368

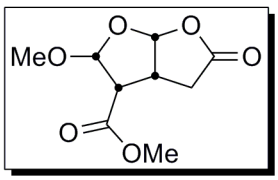
2.41 from Enantioselective Cyclopropanation



PK#	Ret Time	Name	Amount	Amount%	Area	Area%	Type	Width	Height	Height%
2	27.577		0.0000	0.000	1397901.0	16.671	BV	1.096	21261.34	16.197
3	29.779		0.0000	0.000	6757610.0	80.589	VB	1.375	81897.75	62.392

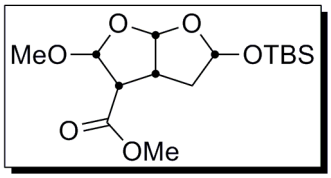


Dihydrofurofuranone 2.63: Cyclopropane **2.41** (0.40 g, 1.86 mmol) was dissolved in benzene (100 mL) and placed in a 350 mL heavy walled pressure vessel. The vessel was sealed and heated to 185 ºC for 24 h, at which time the reaction mixture was allowed to cool to room temperature. The reaction mixture was then concentrated to give a light tan solid that was used without further purification (3.25 g, 82%). $\mathbf{R_f}$ = 0.21 (1:1 hexanes:EtOAc); $\mathbf{[\alpha]_D^{20}}$ = +30.5 (*c* 0.40, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2953, 1797, 1713, 1679, 1645, 1472, 1396, 1282, 1269, 1088, 974 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 6.28 (d, *J* = 6.8 Hz, 1H), 4.01 (m, 1H), 4.00 (s, 3H), 3.70 (s, 3H), 2.85 (d, *J* = 2.8 Hz, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 174.0, 165.3, 164.4, 103.5, 79.4 58.0, 51.2, 41.7, 33.7; **HRMS**-DART (*m/z*) calcd for $C_9H_{11}O_6$ [M^+] 215.0555 found 215.0564; **m.p.** 108 – 112 °C.



Tetrahydrofurofuranone 2.69: Pd/C(0.79 g, 10 wt%, 0.78 mmol) was weighed into a bomb sleeve. EtOAc (20 mL) and Et_3N (1.1 mL, 7.84 mmol) were added, followed by ester **2.63** (1.7 g, 7.84 mmol) in EtOAc (20 mL). The reaction mixture was placed into a Parr bomb, which was sealed, flushed with H_2 and pressurized to 60 atm with H_2. The reaction mixture was allowed to vigorously stir for three days at room temperature, at which point the reaction was judged to be complete by ^{1}H NMR of an aliquot. The reaction mixture was filtered through celite with EtOAc (100 mL) and concentrated. The crude oil was purified on a base washed silica gel column (2:1 hexanes:EtOAc) to give an oil (1.1 g, 63 %) that solidified upon freezing (–20 ºC). $\mathbf{R_f}$ = 0.41 (1:1 hexanes:EtOAc); $\mathbf{[\alpha]_D^{20}}$ = –9.6 (*c* 0.25,

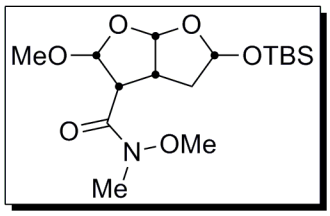
CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 3008, 2953, 2844, 1784, 1738, 1442, 1358, 1236, 1177, 1067, 999, 957 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 6.07 (d, *J* = 6.0 Hz, 1H), 5.25 (d, *J* = 4.4 Hz, 1H), 3.74 (s, 3H), 3.36 (s, 3H), 3.33 (m, 1H), 3.23 (dd, *J* = 8.4, 4.4 Hz, 1H), 3.1 (dd, *J* = 19.2, 5.2 Hz, 1H), 2.74 (dd, 19.2, 11.2 Hz, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 175.6, 168.0, 106.9, 104.7, 55.7, 52.3, 52.1, 37.94, 32.0; **HRMS**-DART (*m/z*) calculated for $C_9H_{13}O_6$ [M^+] 217.0712 found: 217.0720.



TBS protected tetrahydrofurofuranone 2.70: Lactone **2.69** (1.04 g, 4.8 mmol) in THF (240 mL) was cooled to –65 ºC and DIBAL (2.05 mL, 11.5 mmol) was added drop-wise over 15 min. The reaction mixture was warmed to –50 ºC and allowed to stir for 2.5 h, at which time the reaction was judged complete by TLC analysis. Et_2O (80 mL) and saturated aqueous Rochelle's salt (80 mL) were added and the reaction was allowed to warm to room temperature and then stir vigorously for 1 h. The layers were separated and the organic layer was washed with saturated aqueous $NaHCO_3$ (1 x 25 mL) and brine (1 x 25 mL). The three aqueous layers were each extracted with Et_2O (1 x 20 mL, each). The combine organic layers were dried with $MgSO_4$ and concentrated.

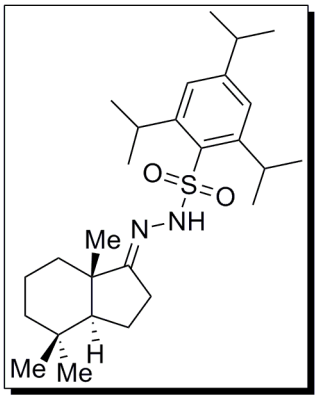
The resulting oil (0.71 g, 3.3 mmol) was dissolved in DMF (3.25 mL) and added to TBSCl (0.76 g, 4.9 mmol) and imidazole (0.44 g, 6.5 mmol). The reaction mixture was allowed to stir for 16 h at room temperature. The reaction mixture was then diluted with EtOAc (10 mL) and H_2O (10 mL), and AcOH (10 drops) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The

combined organic layers were dried with $MgSO_4$ and concentrated. The resulting crude yellow oil was purified on base washed silica gel (3:1 hexanes:EtOAc) to give a pure yellow oil (0.58 g, 48%). $\mathbf{R_f}$ = 0.69 (2:1 hexanes:EtOAc); $\mathbf{[\alpha]_D^{20}}$ = –32.5 (*c* 0.40, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2953, 2928, 2860, 1750, 1442, 1362, 1260, 1210, 1100, 978, 830, 775 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.85 (d, *J* = 5.2 Hz, 1H), 5.61 (dd, *J* = 4.8, 2.0 Hz, 1H), 5.11 (d, *J* = 5.2Hz, 1H), 3.73 (m, 1H), 3.72 (s, 3H), 3.35 (s, 3H), 2.51 (ddd, *J* = 5.6, 7.2, 12.8 Hz, 1H), 2.06 (m, 1H), 0.87 (s, 9H), 0.10 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 169.0, 117.1, 109.2, 104.4, 101.3, 55.6, 52.0, 40.9, 37.9, 25.9, 18.1, -4.2, -5.0; **HRMS**-DART (*m/z*) calcd for $C_{15}H_{28}O_6SiNa$ $[M+Na]^+$ 355.1553, found 355.1554.



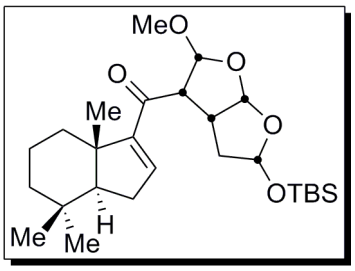
Weinreb amide 2.71: Methyl ester **2.70** (0.49 g, 1.46 mmol) was added in THF (2.9 mL) to N,O-dimethylhydroxylamine hydrochloride (0.22 g, 2.26 mmol) in a 25 mL Schlenk flask. The reaction mixture was cooled to –15 ºC and *i*-PrMgCl (2.6 mL, 4.38 mmol, 1.7 M) was added dropwise over 15 min. The reaction mixture was allowed to stir at –15 ºC for 1.5 h, at which time the reaction was judged complete by TLC analysis. A 50% saturated aqueous solution of NH_4Cl (8 mL) was added and the reaction was allowed to warm to room temperature with stirring. Et_2O (10 mL) was added and the layers were separated. The aqueous layer was extracted with Et_2O (3 x 10 mL), and the combined organic layers dried with $MgSO_4$ and concentrated. The crude yellow oil was purified immediately on base washed silica gel (3:1 hexanes:EtOAc) to give a yellow oil (0.28 g, 53%). $\mathbf{R_f}$ = 0.55 (1:1 hexanes:EtOAc); $\mathbf{[\alpha]_D^{20}}$ = –51.0 (*c* 0.20,

CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2960, 2933, 2855, 1669, 1466, 1381, 1242, 1174, 1101, 1073, 1023, 927, 836 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.81 (d, *J* = 5.6 Hz, 1H), 5.61 (dd, *J* = 4.8, 1.6 Hz, 1H), 5.21 (d, *J* = 4.8 Hz, 1H), 3.68 (s, 3H), 3.34 (m, 1H) 3.33 (s, 3H), 3.18 (s, 3H), 3.17 (m, 1H) 2.68 (ddd, *J* = 13.2, 8.4, 4.8 Hz, 1H), 2.18 (ddd, *J* = 13.6, 9.6, 2.0 Hz, 1H), 0.86 (s, 9H), 0.09 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 169.3, 108.5, 104.0, 101.7, 61.2, 55.3, 50.6, 42.0, 37.9, 32.5, 25.9, 18.2, -4.2, -5.0; **HRMS**-DART (*m/z*) calcd for $C_{16}H_{31}NO_6SiNa$ $[M+Na]^+$ 384.1818, found 384.1811.



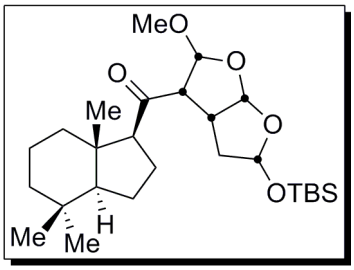
2,4,6-triisopropylphenylsulfonyl hydrazone 2.72: Ketone **2.48** (0.26 g, 1.4 mmol) in CH_3CN (2.3 mL) was added to 2,4,6-triisopropylbenzenesulfonyl hydrazide (0.43 g, 1.4 mmol). The reaction mixture was allowed to stir 15 min at room temperature, and then HBF_4 (3 drops) was added. The reaction mixture was allowed to stir overnight at room temperature, then concentrated. The resulting white solid was taken up in a minimal amount of MeOH. Water was added until the solution was cloudy, and the product was allowed to recrystallize at –20 ˚C. The solid was filtered and dried over P_2O_5 overnight to give a white, flocculent solid (0.41 g, 64%). $\mathbf{R_f}$ = 0.71 (1:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = +37.1 (*c* 0.90, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 3228, 2962, 2928, 2869, 1560, 1476, 1324, 1168, 915, 725, 662 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 7.14 (s, 2H), 7.04 (br s, 1H), 4.21 (quint, *J* = 6.8 Hz, 2H), 2.90 (quint, *J* = 6.8 Hz, 1H), 2.23 (dd, *J* = 18.0, 9.2 Hz, 1H), 2.10 (m, 1H), 1.81 (m, 1H), 1.70 (dt, *J* = 13.6, 3.2 Hz, 1H), 1.54 (m, 4H), 1.42 (dt, *J* = 13.2,

3.6 Hz, 1H), 1.26 (m, 18H), 1.12 (dd, *J* = 13.2, 5.6 Hz, 1H), 1.03 (td, *J* = 13.2, 4.8 Hz, 1H), 0.86 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 170.8, 153.1, 151.4, 123.6, 56.2, 45.3, 41.6, 34.8, 34.3, 33.6, 32.8, 30.0, 25.1, 25.0, 23.8, 21.3, 20.8, 19.6, 19.1; **HRMS**-DART (*m/z*) calcd for $C_{27}H_{44}N_2O_2SNa$ $[M+Na]^+$ 483.3021, found 483.3017; **m.p.** = 154-159 ºC (dec).



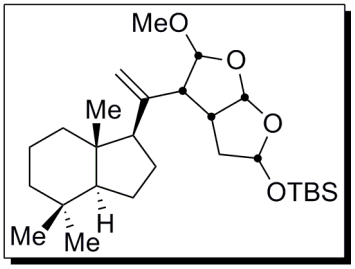
Enone 2.73: Hydrazone **2.72** (0.3 g, 0.651 mmol) in THF (3.25 mL) was added to a 10 mL Schlenk flask and cooled to –78 ºC. *n*Butyllithium (0.9 mL, 1.6 M Et_2O, 1.43 mmol) was added dropwise to the reaction mixture, which was a deep red at the end of the addition. This was allowed to stir at –78 ºC for 1 h, at which point it was warmed to 0 ºC and N_2 evolution took place. The resulting yellow reaction mixture was allowed to stir for 5 min at 0 ºC and then added via cannula to a solution of Weinreb amide (0.107 g, 0.269 mmol) in THF (3.7 mL) at –78 ºC in a 25 mL Schlenk flask. The reaction mixture was warmed to –30 ºC and allowed to stir for 12 h. The reaction was then treated with H_2O (5 mL) and warmed to room temperature. The reaction mixture was diluted with Et_2O (5 mL) and the layers were separated. The aqueous layer was extracted with Et_2O (3 x 5 mL). The combined organic layers were dried with $MgSO_4$ and concentrated to give a viscous orange oil that was purified on base washed silica gel (15:1 hexanes:Et_2O) to give a yellow oil (0.125 g, 99%). $\mathbf{R_f}$ = 0.37 (4:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = –10.4 (*c* 1.0, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2929, 2855, 1743, 1678, 1591, 1471, 1369, 1258, 1109, 1027, 961, 834, 776 cm^{-1}; **^{1}H NMR** (400 MHz,

$CDCl_3$, δ) 6.62 [6.53] (dd, *J* = 3.2, 2.0 Hz, 1H), 5.83 (dd, *J* = 5.6, 1.6 Hz, 1H), 5.61 (td, *J* = 4.4, 2.0 Hz, 1H), 5.15 [5.06] (d, *J* = 4.8 Hz, 1H), 3.60 (dd, *J* = 7.6, 4.8 Hz, 1H), 3.30 [3.28] (s, 3H), 3.13 (m, 1H), 2.58 (m, 1H), 2.35-2.05 (m, 3H), 1.71 (qt, *J* = 13.6, 3.6, 1H), 1.60-1.02 (m, 6H), 0.96 (s, 3H), 0.95 (s, 3H), 0.89 (s, 3H), 0.87 (s, 9 H), 0.09 (s, 6 H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 193.9, 193.5, 156.5, 156.2, 141.9, 141.8, 109.4, 109.1, 106.0, 105.6, 101.9, 59.5, 59.4, 55.9, 55.7, 50.9, 47.9, 47.3, 41.5, 41.1, 37.5, 34.7, 34.5, 33.3, 33.0, 30.2, 26.7, 24.1, 21.4, 21.3, 20.1, 20.0, 18.1, 18.0, 15.5, -4.2, -5.0; **HRMS**-DART (*m/z*) calcd for $C_{25}H_{41}O_4Si$ $[M\text{-}OMe]^+$ 433.2798, found 433.2777.



Ketone 2.74: Enone **2.73** (66 mg, 0.14 mmol) and rhodium on alumina (30 mg, 5 wt%, 0.014mmol) were diluted with EtOAc (4.7 mL) under N_2. This was quickly transferred to a Parr bomb under a blanket of N_2, flushed with H_2 and pressurized to 20 atm with H_2. The reaction was allowed to vigorously stir for 18 h at room temperature, at which point it was filtered through celite with EtOAc (10 mL) and concentrated. The crude oil was purified on base washed silica gel (15:1 hexanes:Et_2O) to provide a clear oil (0.43 g, 65%). $\mathbf{R_f}$ = 0.35 (6:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = +12.8 (*c* 1.25, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2951, 2922, 2858, 1736, 1703, 1456, 1367, 1246, 1193, 1104, 1088, 996, 832 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.81 (d, *J* = 4.8 Hz, 1H), 5.57 (dd, *J* = 4.8, 1.6 Hz, 1H), 5.21 (d, *J* = 5.2 Hz, 1H), 3.34 (s, 3H), 3.13 (ddd, *J* = 16.4, 13.6, 8.0 Hz, 1H), 3.07 (dd, *J* = 7.6, 4.8 Hz, 1H), 2.54 (t, *J* = 8.8 Hz, 1H), 2.35 (ddd, *J* = 13.2, 8.0, 5.2 Hz, 1H), 2.16 (ddd, *J* = 11.2, 9.2, 1.6 Hz, 2H), 1.84 (dt, *J* = 11.6, 4.0

Hz, 1H), 1.76-1.16 (m, 8H), 1.09 (td, J = 13.6, 4.4 Hz, 1H), 0.88 (s, 3H), 0.87 (s, 9H), 0.86 (s, 3H), 0.68 (s, 3H), 0.09 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 232.6, 109.3, 104.8, 101.6, 63.7, 60.7, 59.3, 55.5, 44.9, 41.4, 41.0, 40.3, 37.9, 33.6, 25.9, 22.9, 22.1, 21.2, 20.9, 20.1, 18.1, 15.0, -4.2, -5.0; **HRMS**-DART (*m/z*) calculated for $C_{26}H_{46}O_5SiNa$ $[M+Na]^+$ 489.3012, found 489.3011

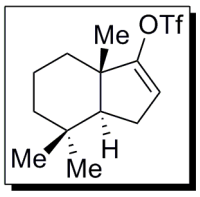


1,1-Disubstituted olefin 2.83: $TMSCH_2MgCl$ was prepared according to the following procedure: To magnesium (0.12 g, 4.98 mmol) was added $TMSCH_2Cl$ (0.6 mL, 4.1 mmol) in Et_2O (2.4 mL). The reaction mixture was heated to reflux for 1 h and then allowed to cool to room temperature, at which time titration showed the resulting solution to be 1 M (57%). The solution was used immediately in the subsequent reaction.

To a solution of ketone **2.80** (0.019 g, 0.041 mmol) in Et_2O (0.6 mL) was added $TMSCH_2MgCl$ (0.41 mL, 1 M Et_2O, 0.41 mmol). The reaction mixture was allowed to stir 16 h at room temperature at which time the reaction was treated with H_2O (5 mL) and AcOH (5 drops). The layers were separated and the aqueous layer was extracted with Et_2O (3 x 2 mL), and the combined organic layers dried with $MgSO_4$ and concentrated to give a clear, colorless oil (0.021 g, 93%) that did not require further purification.

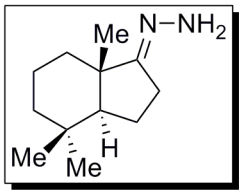
To a solution of hydroxyl silane **2.81** (12 mg, 0.022 mmol) in THF (0.6 mL) was added KHMDS (8 mg, 0.043 mmol) in THF (0.6 mL). The resulting orange reaction mixture was allowed to stir for 15 min at room temperature. Pyridine (14 μL, 0.17

mmol) and triflic anhydride (8 μL, 0.043 mmol) were then added and the resulting red reaction mixture was allowed to stir for 2 h, at which time the reaction was judged complete by TLC analysis (100% CH_2Cl_2). Saturated aqueous sodium bicarbonate (1 mL) and Et_2O (1 mL) were added and the layers separated. The aqueous layer was extracted with Et_2O (3 x 1 mL). The combined organic layers were dried with $MgSO_4$ and concentrated to give a crude orange oil. The crude oil was purified on base washed silica gel (20:1 hexanes:Et_2O) to give a clear, colorless oil (7.5 mg, 75%). $\mathbf{R_f}$ = 0.66 (5:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = +1.1 (*c* 0.375, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2947, 2923, 2856, 2363, 2343, 1250, 1101, 988, 831 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.85 (d, *J* = 5.6 Hz, 1H), 5.57 (dd, *J* = 5.2, 2.0 Hz, 1H), 5.09 (s, 1H), 5.03 (s, 1H), 5.01 (d, *J* = 4.4 Hz, 1H), 3.34 (s, 3H), 3.12 (m, 1 H), 2.88 (td, *J* = 3.6, 2.0 Hz, 1H), 2.50 (ddd, *J* = 13.2, 8.0, 5.6 Hz, 1H), 2.05 (dd, *J* = 18.0, 8.8 Hz, 1H), 1.89 (ddd, *J* = 12.4, 9.6, 2.4 Hz, 1H), 1.76-0.95 (m, 9H), 0.88 (s, 9H), 0.86 (s, 6H), 0.66 (s, 3H), 0.11 (s, 3H), 0.11 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 143.6, 114.0, 108.9, 105.5, 101.2, 59.1, 56.4, 55.4, 53.0, 43.3, 42.4, 41.7, 40.0, 37.5, 33.4, 26.0, 25.2, 20.9, 20.8, 20.3, 18.2, 14.0, -4.1, -4.9; **HRMS**-DART (*m/z*) calcd for $C_{26}H_{45}O_3Si$ $[M\text{-}OMe]^+$ 433.3138, found 433.3143.



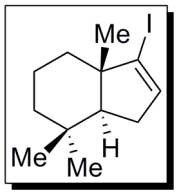
Hydrindane triflate 2.84: Hydrindane ketone **2.48** (50 mg, 0.28 mmol) was dissolved in THF (4.0 mL) in a round bottom flask and cooled to –78 °C. LDA (0.61 mL, 0.31 mmol) was added drop-wise and the reaction mixture was allowed to stir at -78 °C for 15 min. Comins reagent (0.12 g, 0.31 mmol) in THF (0.6mL) was added and the reaction mixture was allowed to stir

and warm to room temperature overnight, at which point it was judged complete by TLC analysis. The reaction mixture was diluted with Et_2O (5 mL) and saturated aqueous ammonium chloride (5 mL) was added. The layers were separated and the aqueous layers were extracted with diethyl ether (3 x 5 mL). The combined organic layers were then washed with 5% aqueous NaOH (2 x 5 mL), dried with $MgSO_4$, filtered and concentrated. The crude orange oil was purified on a base washed silica gel column (20:1 hexanes:Et_2O) to provide a clear oil (53 mg, 61%). $\mathbf{R_f}$ = 0.35 (hexanes); $\mathbf{[\alpha]_D^{20}}$ = –8.2 (*c* 0.17, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$2940, 2863, 1632, 1420, 1207, 1412, 1064, 1036, 917, 868, 856; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.59 (dd, *J* = 3.6, 2.0 Hz, 1H), 2.19 (ddd, *J* = 14.8, 6.4, 3.2 Hz, 1H), 2.11 (ddd, *J* = 14.8, 11.2, 2.0 Hz, 1H), 1.66 (m, 4 H), 1.49 (dt, *J* = 12.4, 3.2, 1H), 1.35 (m, 1H), 1.16 (td, *J* = 13.2, 4.8 Hz, 1H), 1.07 (s, 3 H), 0.96 (s, 3H), 0.90 (s, 3H); **^{13}C NMR** (100 MHz $CDCl_3$, δ) 159.0, 118.8 (q, $J_{19F\text{-}13C}$ 340 Hz), 114.2, 57.4, 45.5, 41.4, 33.4, 32.9, 32.3, 26.6, 21.4, 19.6, 17.4; **HRMS**-DART (*m/z*) calcd for $C_{12}H_{19}[M\text{-}OTf]^+$ 163.1486, found 163.14868.

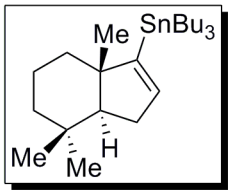


Hydrazone 2.85: A mixture of ketone **2.48** (0.31 g, 0.17 mmol), Et_3N (2.06 mL, 1.48 mmol) and hydrazine hydrate (0.67 mL, 1.38 mmol) in EtOH (5.4 mL) was heated to reflux for 16 h. The reaction was then cooled to room temperature and water (25 mL) was added to produce a white precipitate, which increased upon cooling to 0 ºC. The reaction mixture was then filtered to give a white flocculent solid (0.28 g, 84%). **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 4.70 (br s, 2H), 2.26 (ddd, *J* = 18.0, 9.2, 1.6 Hz, 1H), 2.15 (quint, *J* = 9.2 Hz, 1H), 1.86-

1.52 (m, 5H), 1.46 (ddt, *J* = 13.6, 6.4, 2.8 Hz, 1H), 1.26 (td, *J* = 12.8, 4.4 Hz, 1H), 1.17 (dd, *J* = 13.6, 6.0 Hz, 1H), 1.09 (td, *J* = 13.6, 4.4 Hz, 1H), 0.96 (s, 3H), 0.90 (s, 6H).

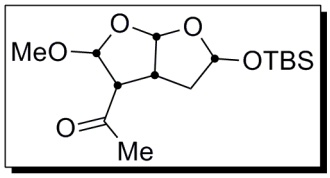


Vinyl iodide 2.86: Triethylamine (1.8 mL, 1.30 mmol) was added to a solution of hydrazone **2.85** (0.28 g, 0.14 mmol) in tetrahydrofuran (8.5 mL). Iodine dissolved in THF was added until a brown color persisted in the reaction mixture. The reaction was allowed to stir for 5 min. The reaction mixture was diluted with ether (10 mL) and washed with: 1 M HCl (2 x 5 mL); saturated, aqueous $Na_2S_2O_3$ (2 x 5 mL); and saturated, aqueous $NaHCO_3$ (2 x 5 mL). The organic layer was then dried with $MgSO_4$ and concentrated. The crude reaction mixture was purified by silica gel chromatography (100% hexanes) to provide a pale brown oil (0.29 g, 70%). **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 6.15 (t, *J* = 2.8 Hz, 1H), 2.08 (dd, *J* = 9.6, 2.4 Hz, 2H), 1.70-1.53 (m, 3H), 1.47 (ddt, *J* = 19.2, 12.4, 3.2, 2H), 1.16-1.05 (m, 2H), 0.95 (s, 3H), 0.91 (s, 3H), 0.82 (s, 3H).



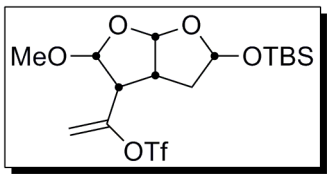
Hydrindane stannane 2.88: *t*BuLi (0.18 mL, 1.9 M in pentane, 0.31 mmol) was added dropwise to a solution of vinyl iodide **2.86** (42 mg, 0.145 mmol) in Et_2O (2.4 mL) at –78 ºC, and the reaction mixture was allowed to stir for 45 min. Bu_3SnCl (47 µL, 0.174 mmol) was added and the reaction mixture was allowed to stir and warm to room temperature over 16 h, at which point it was washed with saturated aqueous NH_4Cl (2 x 1 mL) and saturated aqueous $NaHCO_3$ (2 x 1 mL) and dried with $MgSO_4$ and concentrated. The resulting clear oil was pumped on

high vacuum for 4 h and then purified via silica gel chromatography (100% hexanes) (24 mg, 42%). $\mathbf{R}_f$ = 0.9 (hexanes); $\mathbf{[\alpha]_D^{20}}$ = +0.2 (*c* 5.0, CH_2Cl_2). **IR** (thin film) $\bar{\nu}_{max}$ 2953, 2920, 2843, 1461 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.88 (dd, *J* = 4.0, 2.0 Hz, 1H), 2.14 (ddd, *J* = 14.8, 11.6, 1.2 Hz, 1H), 2.06 (ddd, *J* = 15.2, 6.4, 3.2 Hz, 1H), 1.78-1.54 (m, 4H), 1.53-1.42 (m, 12H), 1.31 (td, *J* = 14.4, 7.2, 6H), 1.26-1.11 (m, 3 H), 1.10 (s, 6H), 0.89 (td, *J* = 7.6, 2.4 Hz, 9H), 0.79 (s, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 158.9, 139.4, 59.7, 51.1, 41.9, 38.1, 33.7, 33.2, 31.4, 31.0, 29.6, 29.4, 27.9, 27.6, 21.3, 20.5, 19.1, 13.9, 9.9, 8.1; **HRMS**-DART (*m/z*) calcd for $C_{20}H_{37}Sn$ $[M\text{-}Bu]^+$ 397.1917, found 397.1915.



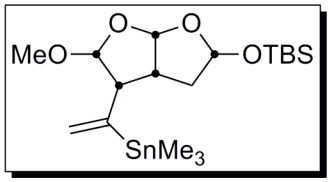
Methyl ketone: To a solution of Weinreb amide **2.71** (0.03 g, 0.083 mmol) in THF (0.6 mL) at 0 ºC was added methyl Grignard (0.06 mL, 0.166 mmol). The reaction mixture was allowed to stir at 0 ºC for 30 min at which point it was judged complete by TLC analysis. Saturated, aqueous ammonium chloride (0.5 mL) was added at 0 ºC, the reaction mixture was warmed to room temperature and extracted with ether (4 x 1 mL). The combined organic layers were dried with $MgSO_4$ and concentrated. The pale orange oil was purified on base washed silica gel chromatography (3:1 hexanes:EtOAc) to provide a colorless oil (0.023 g 88%). $\mathbf{R}_f$ = 0.33 (1:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = –28.2 (*c* 1.10, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2955, 2922, 2860, 1721, 1475, 1360, 1249, 1200, 1106, 983, 835 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.83 (d, *J* = 4.8 Hz, 1H), 5.57 (dd, *J* = 4.8, 2.0 Hz, 1H), 5.24 (d, *J* = 5.2 Hz, 1H), 3.37 (s, 3H), 3.16 (m, 1H), 2.29 (ddd, *J* =

18.4, 13.6, 5.2 Hz, 1H), 2.18 (s, 3H), 2.10 (ddd, *J* = 11.6, 9.6, 2.0 Hz, 1H), 0.86 (s, 9H), 0.09 (s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 202.9, 109.3, 104.1, 101.5, 60.4, 55.4, 40.6, 37.8, 29.7, 25.9, 18.1, -4.2, -5.0; **HRMS**-DART (*m/z*) calcd for $C_{15}H_{28}O_5SiNa$ $[M+Na]^+$ 339.1604, found 339.1599.

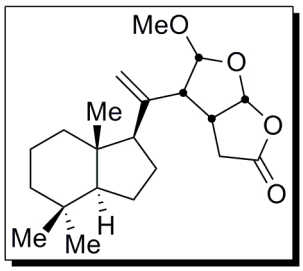


Triflate 2.90: LDA (0.21 mL, 0.5 M in THF, 0.104 mmol) was added to a solution of methyl ketone (30 mg, 0.095 mmol) in THF (1.0 mL) at –78 ºC. After 15 min Comins' reagent (41 mg, 0.104 mmol) was added and the reaction was allowed to stir at -78 ºC for 16 h at which time brine (1 mL) was added and the reaction allowed to warm to room temperature. The reaction mixture was diluted with Et_2O and the layers were separated. The aqueous layer was extracted with Et_2O (2 x 1 mL) and the combined organic layers were washed with 5% aqueous NaOH (3 x 1 mL). The organic layer was then dried with $MgSO_4$ and concentrated. The crude orange oil was purified by a base washed silica gel plug (100% Et_2O) followed by a base washed silica gel column (30:1 hexanes:Et_2O) to provide the pure product as a clear oil (21 mg, 50%). $\mathbf{R_f}$ = 0.45 (5:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = –25.8 (*c* 0.45, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2959, 2928, 2857, 2357, 2338, 1417, 1213, 1144, 965, 928, 830 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.92 (d, *J* = 5.6 Hz, 1H), 5.61 (dd, *J* = 4.8, 2.4 Hz, 1H), 5.34 (s, 2H), 5.03 (d, *J* = 4.4 Hz, 1H), 3.37 (s, 3H), 3.22 (m, 1 H), 3.14 (dd, *J* = 8.0, 4.4 Hz, 1H), 2.37 (ddd, *J* = 12.4, 6.8, 5.2 Hz, 1H), 1.85 (ddd, *J* = 12.4, 9.6, 2.4 Hz, 1H), 0.89 (s, 9H), 0.11(s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 151.5, 118.0 (q, $J_{19F\text{-}13C}$ 318 Hz), 108.9, 108.3, 103.7, 100.9, 55.3, 50.2, 41.8, 36.8, 25.9,

13.1, -4.2, -5.0; **HRMS**-DART (*m/z*) calcd for $C_{16}H_{27}O_7SiSF_3Na$ $[M+Na]^+$ 471.1097, found 471.1080.



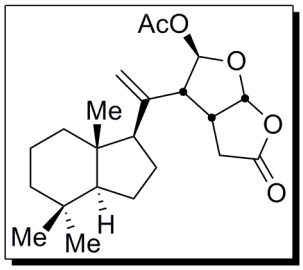
Stannane 2.91: Palladium dibenzylidene acetone (1.5 mg, 0.0013 mmol), lithium chloride (5.0 mg, 0.107 mmol) and triphenyl arsine (1.0 mg, 0.0027 mmol) were weighed out in a glovebox into a vial containing a stirbar, which was sealed with a septum. The vial was removed from the glovebox and THF (0.7 mL) was added, followed by hexamethyl ditin (14.0 μL, 0.067 mmol). Vinyl triflate **2.90** (12.0 mg, 0.0267 mmol) was then added in THF (0.7 mL). The solution was allowed to stir at room temperature for 48 h, at which time it was judged complete by TLC analysis. H_2O (2 mL) was added and the reaction mixture was extracted with Et_2O (3 x 5 mL). The combined organic layers were dried with $MgSO_4$ and concentrated. The crude oil was purified on a base washed silica gel column (20:1 hexanes:Et_2O) to give a colorless oil (5.0 mg, 41%). $\mathbf{R_f}$ = 0.44 (10:1 hexanes: Et_2O); $\mathbf{[\alpha]_D^{20}}$ = –5.2 (*c* 0.267, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2962, 2926, 2853, 2364, 2336, 1476, 1359, 1258, 1092, 979, 830, 773 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 5.87 (d, *J* = 5.6 Hz, 1H), 5.85 (t, *J* = 2.0 Hz, 1H), 5.53 (dd, *J* = 5.2, 3.2 Hz, 1H), 5.48 (t, *J* = 2.4 Hz, 1H), 4.84 (d, *J* = 4.4 Hz, 1H), 3.32 (s, 3H), 3.15 (m, 2H), 2.24 (m, 1H), 1.71 (ddd, *J* = 13.2, 10.4, 3.2 Hz, 1H), 0.88 (s, 9H), 0.10 (s, 9H); **^{13}C NMR** (100 MHz, $CDCl_3$, δ) 150.5, 129.1, 108.5, 105.2, 100.8, 54.5, 54.3, 44.6, 36.8 26.0, 18.2, -4.2, -5.0, -7.6; **HRMS**-DART (*m/z*) calculated for $C_{17}H_{33}O_3SiSn$ $[M-OMe]^+$ 433.1221, found 433.1234.



Lactone 2.93: To TBS protected **2.83** (5 mg, 0.011 mmol) dissolved in THF (0.43 mL) was added acetic acid (6 μL, 0.11 mmol) immediately followed by TBAF (0.11 mL, 1 M in THF, 0.11 mmol). The reaction mixture was allowed to stir 3 h at room temperature, at which point it was judged complete by TLC analysis. The reaction mixture was diluted with Et_2O (0.5 mL) and H_2O (1 mL). The layers were separated and the aqueous layer was extracted with Et_2O (3 x 2 mL). The combined aqueous layers were dried with $MgSO_4$, filtered and concentrated and used immediately.

To the resulting clear oil was added PCC (5 mg, 0.022 mmol), sodium acetate (3 mg, 0.024 mmol), celite (5 mg) and 4 Å molecular sieves (5 mg). CH_2Cl_2 (0.85 mL) was added and the reaction mixture was allowed to stir for 12 h. At the completion of the reaction, Et_2O (3 mL) was added and the mixture was filtered through a base washed silica gel plug with Et_2O and concentrated. The crude oil was purified on base washed silica gel (2:1 hexanes:Et_2O) to provide a clear oil (3 mg, 80%). $\mathbf{R}_f$ = 0.38 (1:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = +4.9 (*c* 0.2, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2922, 2838, 2353, 2333, 1788, 1363, 1174, 1094, 990 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 6.09 (d, *J* = 6.0 Hz, 1H), 5.14 (d, *J* = 4.0 Hz, 1H), 5.11 (s, 1H), 5.01 (d, *J* = 1.6 Hz, 1H), 3.36 (s, 3H), 3.26 (m, 1H), 3.03 (dd, *J* = 18.8, 6.0 Hz, 1H), 2.89 (m, 1H), 2.56 (dd, *J* = 18.4, 10.8 Hz, 1H), 2.03 (t, *J* = 9.6 Hz, 1H), 1.75-1.0 (m, 11H), 0.86 (s, 6H), 0.67 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$, δ) 176.4, 142.8, 115.7, 107.3, 106.3, 59.1, 56.8, 55.6, 53.0, 43.6, 41.7,

39.9, 39.8, 33.6, 33.5, 31.8, 30.6, 25.3, 20.90, 20.87, 20.3; **HRMS**-DART (*m/z*) cald for $C_{20}H_{29}O_3$ $[M\text{-}OMe]^+$ 317.2117, found 317.2118.



Norrisolide 2.1: To methyl ether **2.93** (2.4 mg, 0.0069 mmol) was added water (36 μL) and TFA (0.12 mL). The reaction mixture was allowed to stir for 2 h at room temperature, at which point the reaction was judged complete by TLC analysis. The reaction mixture was then diluted with EtOAc and carefully washed with saturated aqueous $NaHCO_3$ (3 x 1 mL). The combined aqueous layers were extracted with EtOAc (2 x 1 mL) and the combined organic layers were dried with $MgSO_4$, filtered and concentrated. The crude oil was immediately taken on without purification.

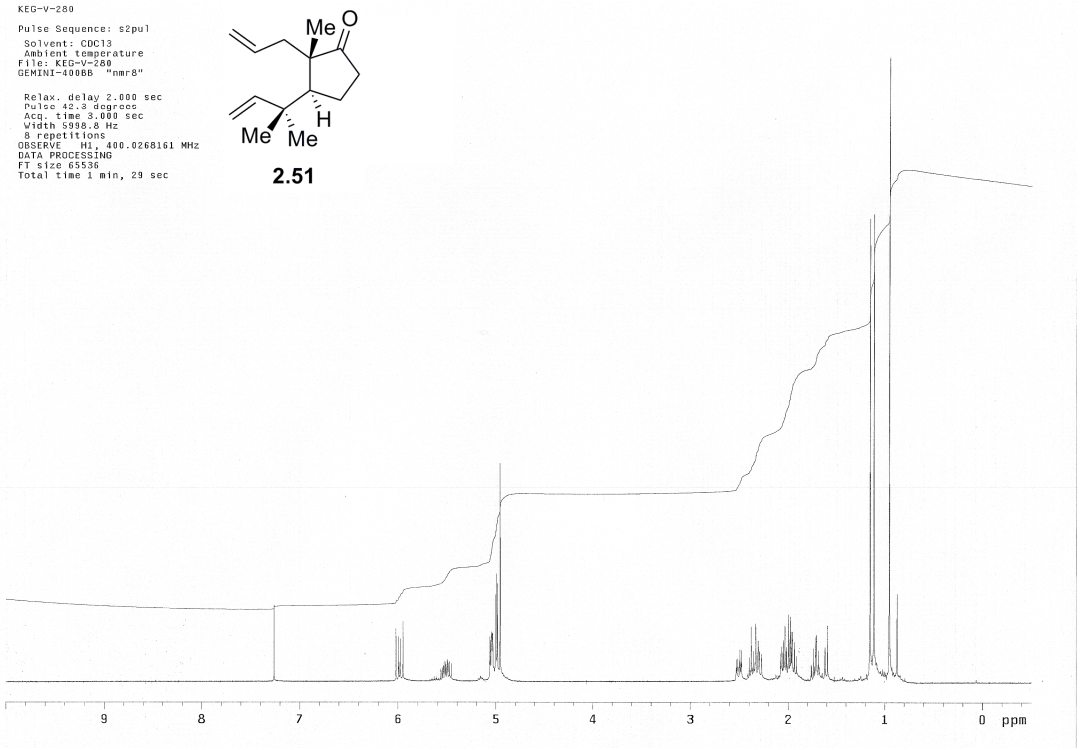
DMAP (0.1 mg, 0.0008 mmol) and the lactol were dissolved in CH_2Cl_2 (1.0 mL). Et_3N (4.8 μL, 0.034 mmol) and acetic anhydride (3.3 μL, 0.034 mmol) were added and the reaction mixture was allowed to stir for 1 h at room temperature. The reaction mixture was then flushed through a plug (1 cm) of base washed silica gel with CH_2Cl_2 (3 mL) and then EtOAc (8 mL) and concentrated. The crude oil was purified on a base washed silica gel (2:1 hexanes:Et_2O) to provide norrisolide (1.0 mg, 40%). $\mathbf{R}_f$ = 0.28 (1:1 hexanes:Et_2O); $\mathbf{[\alpha]_D^{20}}$ = +3.2 (*c* 0.09, CH_2Cl_2); **IR** (thin film) $\bar{\nu}_{max}$ 2922, 2850, 2365, 2333, 1801, 1757, 1220, 1017 cm^{-1}; **^{1}H NMR** (400 MHz, C_6D_6, δ) 6.63 (d, *J* = 4.0 Hz, 1H), 5.66 (d, *J* = 6.4 Hz, 1H), 4.91 (s, 1H), 4.78 (s, 1H), 2.71 (ddd, *J* = 8.8, 3.6, 1.0 Hz), 2.4-2.3 (m, 1H), 2.14 (dd, *J* = 18.4, 4.0 Hz) 1.75 (dd, *J* = 18.4, 10.4 Hz), 1.59 (s, 3H), 1.45-0.6 (m, 12H), 0.86 (s, 3H), 0.80 (s, 3H), 0.47 (s, 3H); **^{1}H NMR** (400 MHz, $CDCl_3$, δ) 6.45 (d, *J* = 3.6 Hz, 1H), 6.14 (d, *J* = 6.0 Hz, 1 H), 5.16 (s, 1H), 5.09 (s, 1H),

3.36 (m, 1H), 3.07 (dd, *J* = 9.6, 3.2, 1H), 2.55 (d, *J* = 7.2 Hz, 2H), 2.20-1.90 (m, 2H), 2.08 (s, 3H), 1.76-1.38 (m, 6H), 1.15-0.96 (m, 4H), 0.86 (s, 3H), 0.85 (s, 3H), 0.67 (s, 3H); **^{13}C NMR** (125 MHz, C_6D_6, δ) 173.3, 168.4, 143.3, 116.8, 107.0, 101.7, 58.6, 57.7, 50.0, 44.9, 41.7, 40.0, 38.5, 33.3, 33.1, 30.4, 24.2, 21.1, 20.6, 20.3, 19.8, 14.1; **HRMS**-DART (*m/z*) cald for $C_{20}H_{29}O_3$ $[M\text{-}OAc]^+$ 317.2117, found 317.2108.

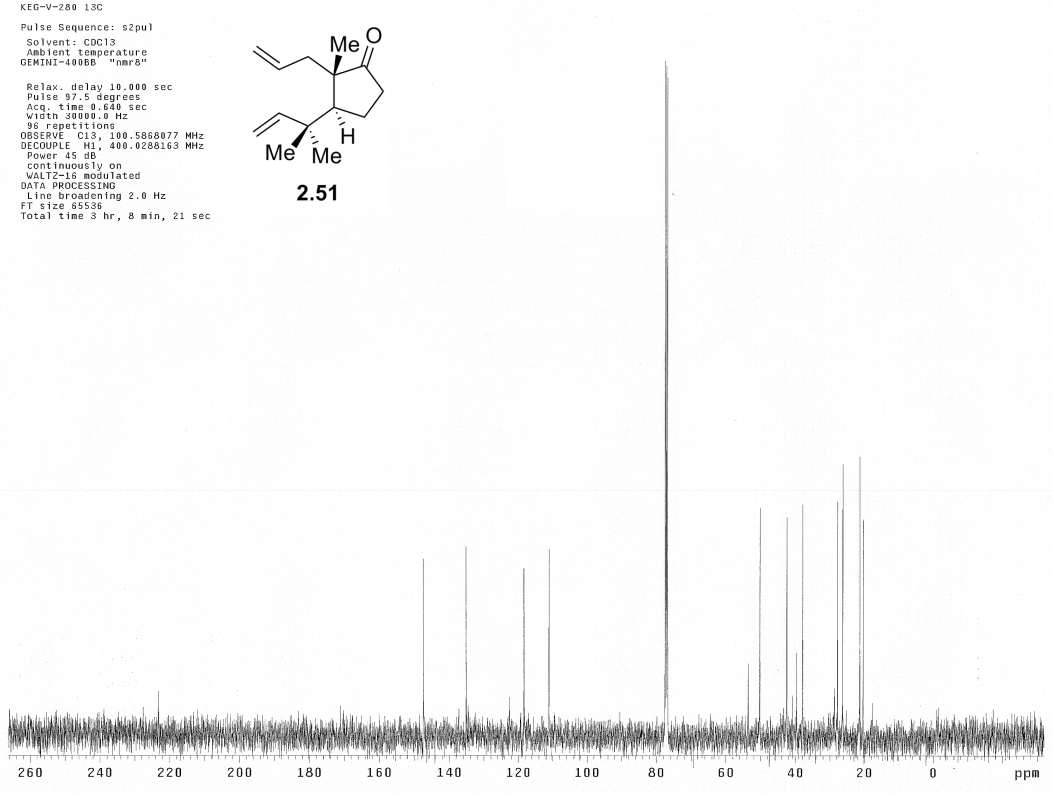
Authentic:[1] **^{1}H NMR** ($CDCl_3$, δ) 6.44 (d, *J* = 3.5 Hz, 1 H), 6.14 (d, *J* = 6.0 Hz, 1 H), 5.15 (br s, 1H), 5.09 (br s, 1H), 3.36 (m, 1H), 3.07 (dd, *J* = 9.5, 3.5 Hz, 1H), 2.55 (d, *J* = 7.0 Hz, 2H), 2.2-1.9 (m, 2 H), 2.07 (s, 3H), 1.75-1.35 (m, 6H), 1.1-0.9 (m, 4H) 0.86 (s, 3H), 0.84 (s, 3H), 0.66 (s, 3H); **^{13}C NMR** (C_6D_6, δ) 173.6, 168.6, 143.5, 116.8, 107.1, 101.8, 58.8, 57.8, 50.1, 45.1, 41.8, 40.6, 38.7, 33.5, 33.3, 30.5, 24.3, 21.2, 20.7, 20.5, 19.9, 14.2

[1] Faulkner, D. J.; Hochlowski, J. E. *J. Org. Chem.* **1983**, *48*, 1141-1142.

^{1}H NMR 2.51



^{13}C NMR 2.51



^{1}H NMR 2.52

KEG-V-282

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Ambient temperature
GEMINI-400BB "nmr8"

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Pulse 42.3 degrees
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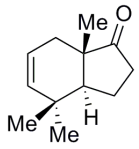
Width 5998.8 Hz
4 repetitions
OBSERVE H1, 400.0268158 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



2.52

^{13}C NMR 2.52

KEG-V-282-13C

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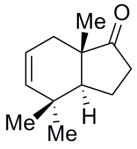
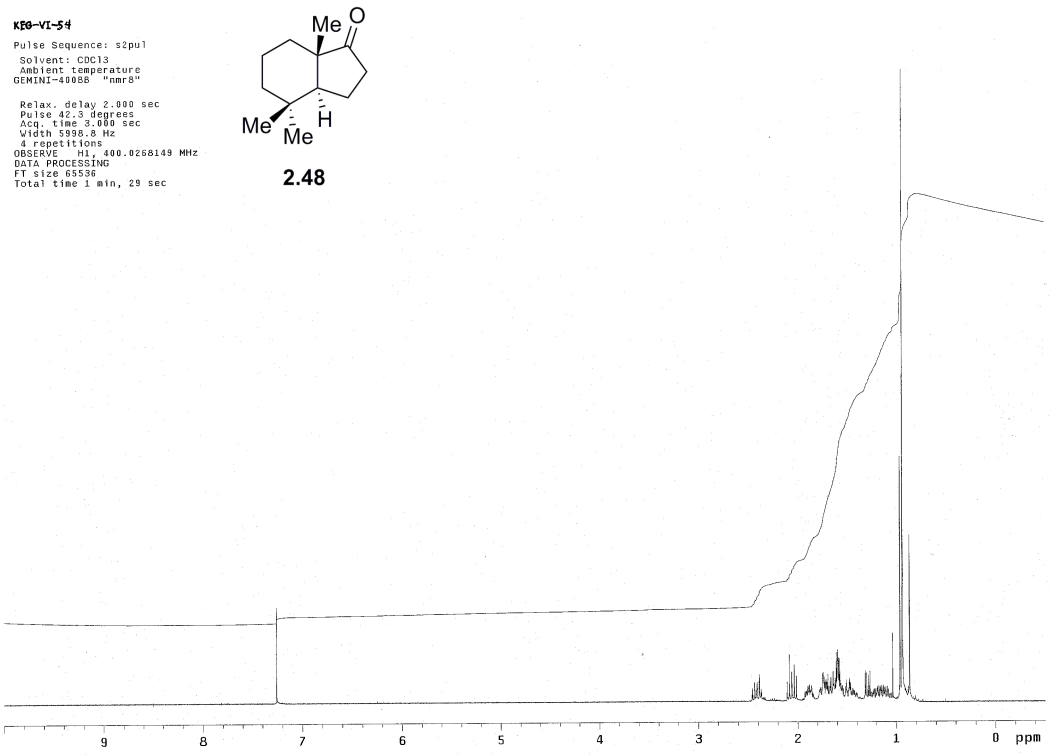
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 10.000 sec
Pulse 97.5 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
120 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 3 hr, 8 min, 21 sec



2.52

^{1}H NMR 2.48


KEG-VI-54
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"
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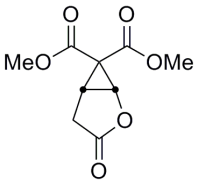
Pulse 42.3 degrees
Acq. time 3.000 sec
Width 5995.8 Hz
4 repetitions
OBSERVE H1, 400.0268149 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec
Me
O
Me
H
Me
2.48
9
8
7
6
5
4
3
2
1
0
ppm

^{1}H 2.41

KEG-VI-14

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 42.3 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
8 repetitions
OBSERVE H1, 400.0268154 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



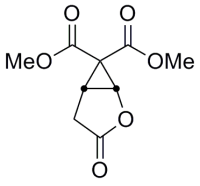
2.41

^{13}C NMR 2.41

13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 4.000 sec
Pulse 97.5 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
192 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 1 hr, 10 min, 41 sec



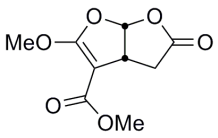
2.41

^{1}H NMR 2.63

KEG-V-197

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 45.6 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
16 repetitions
OBSERVE H1, 400.0268160 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



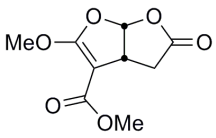
2.63

^{13}C NMR 2.63

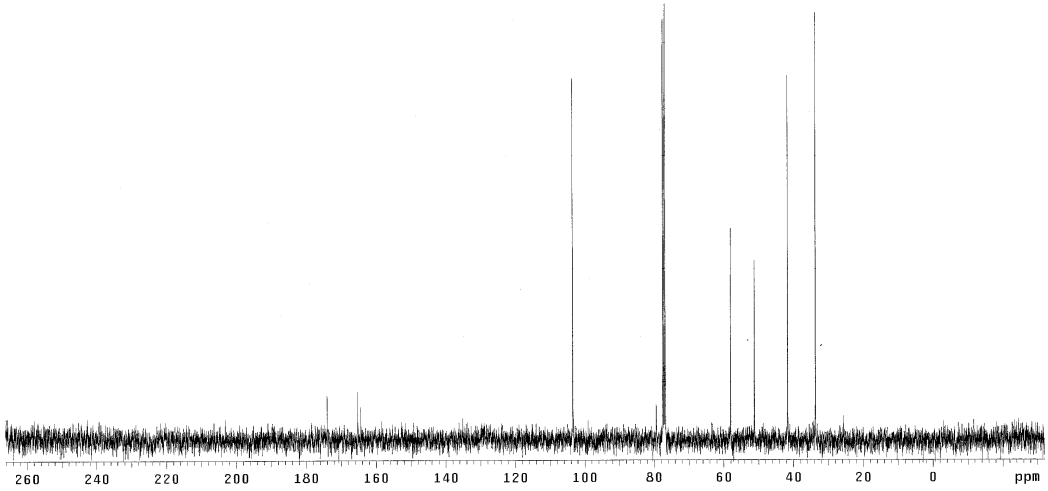
KEG-V-197 13C

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

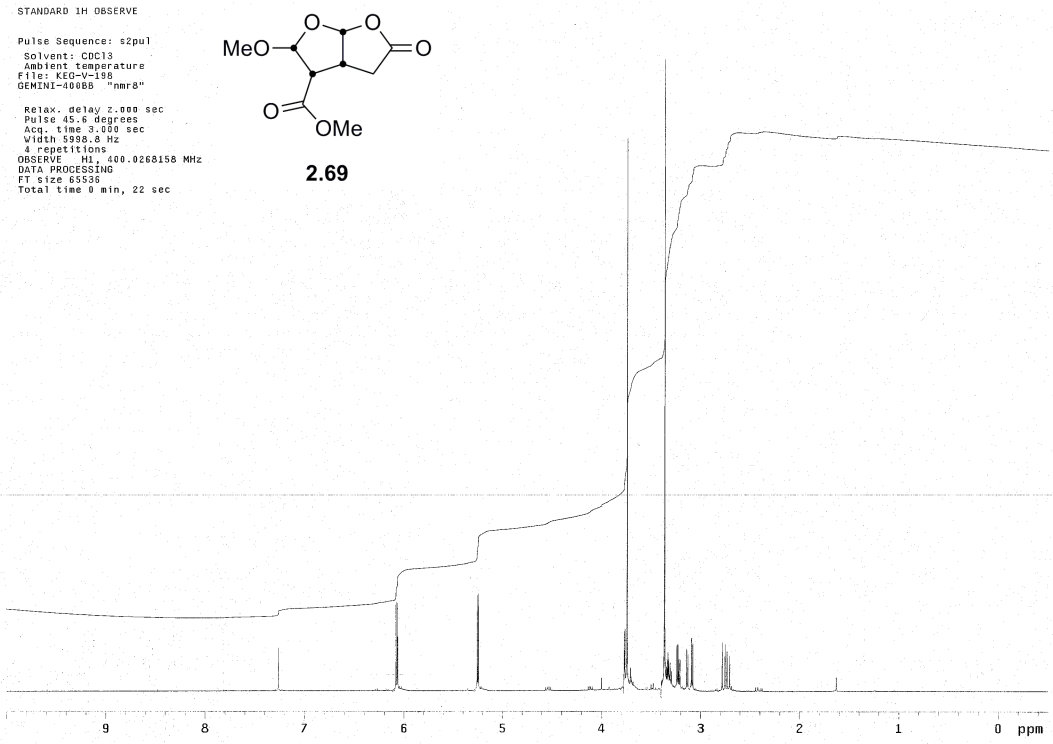
Relax. delay 4.000 sec
Pulse 81.2 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
176 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 35 min, 20 sec



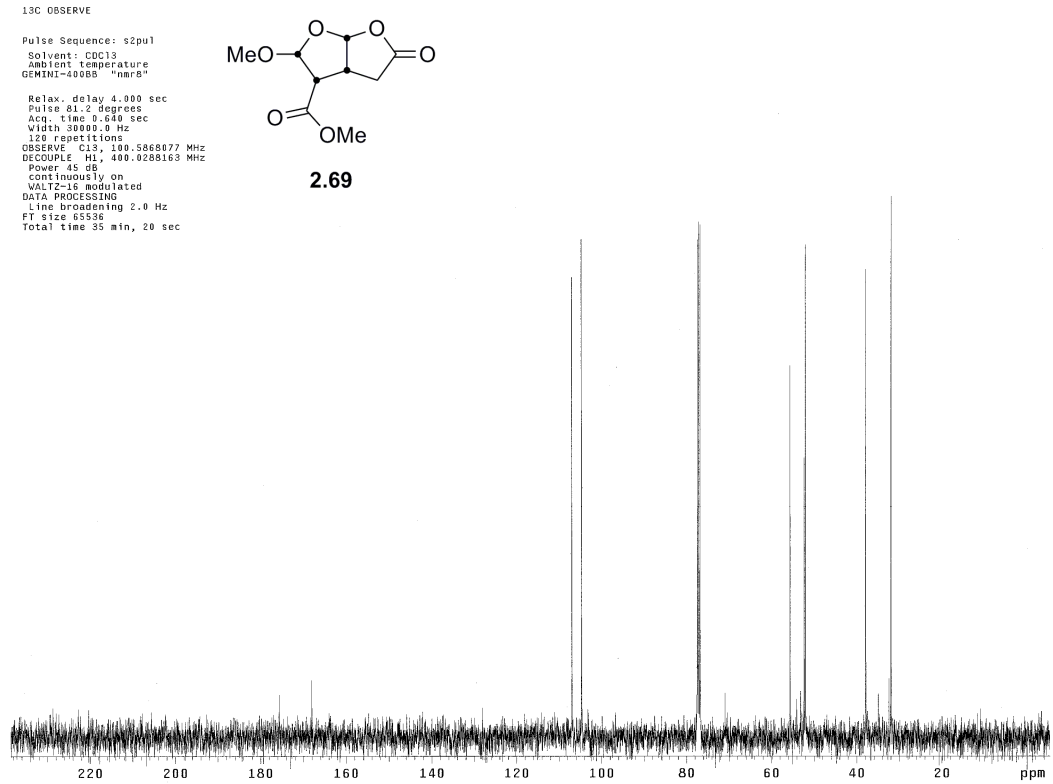
2.63



^{1}H NMR 2.69



^{13}C NMR 2.69

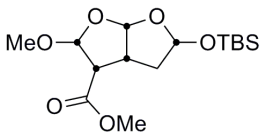


^{1}H NMR 2.70

KEG-V-200

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 45.6 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
4 repetitions
OBSERVE H1, 400.0268158 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec



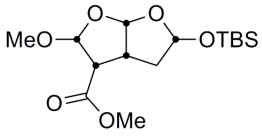
2.70

^{13}C NMR 2.70

13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 4.000 sec
Pulse 81.2 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
92 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 35 min, 20 sec



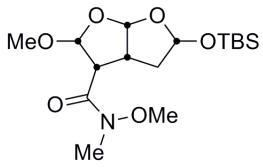
2.70

^{1}H NMR 2.71

KEG-V-201

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 45.6 degrees
Acq. time 3.000 sec
Width 3998.6 Hz
4 repetitions
OBSERVE H1, 400.0268152 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 22 sec



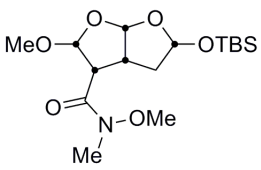
2.71

^{13}C NMR 2.71

13C OBSERVE

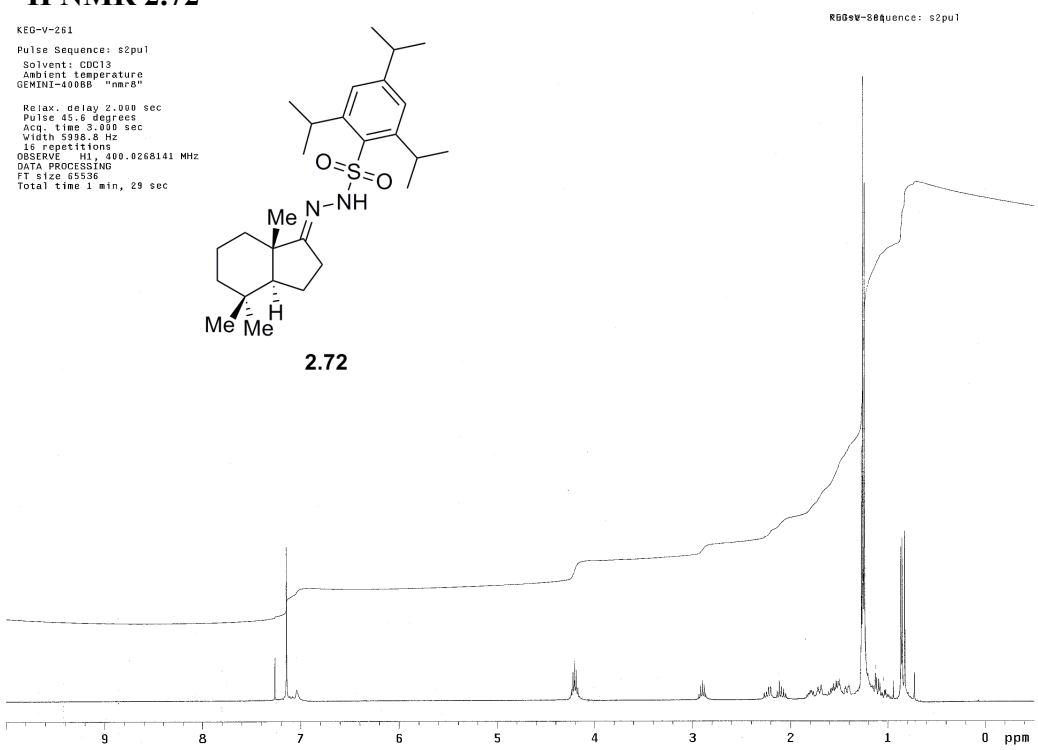
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 4.000 sec
Pulse 81.2 degrees
Acq. time 0.640 sec
Width 25603.4 Hz
68 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0268163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 17 min, 23 sec



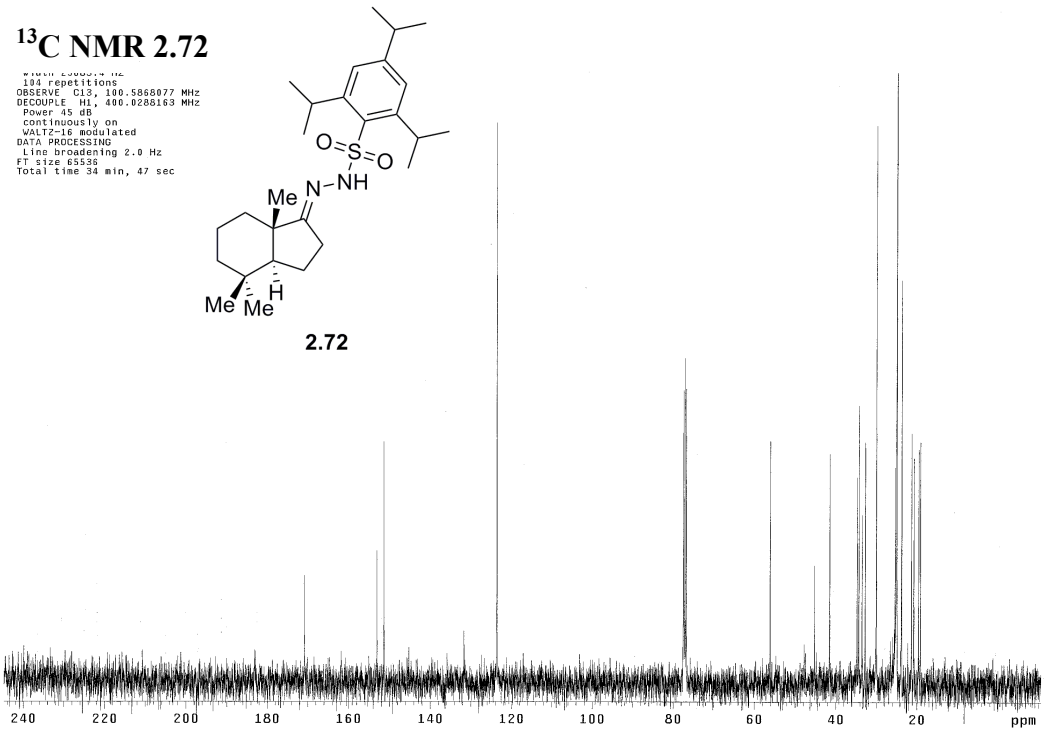
2.71

^{1}H NMR 2.72



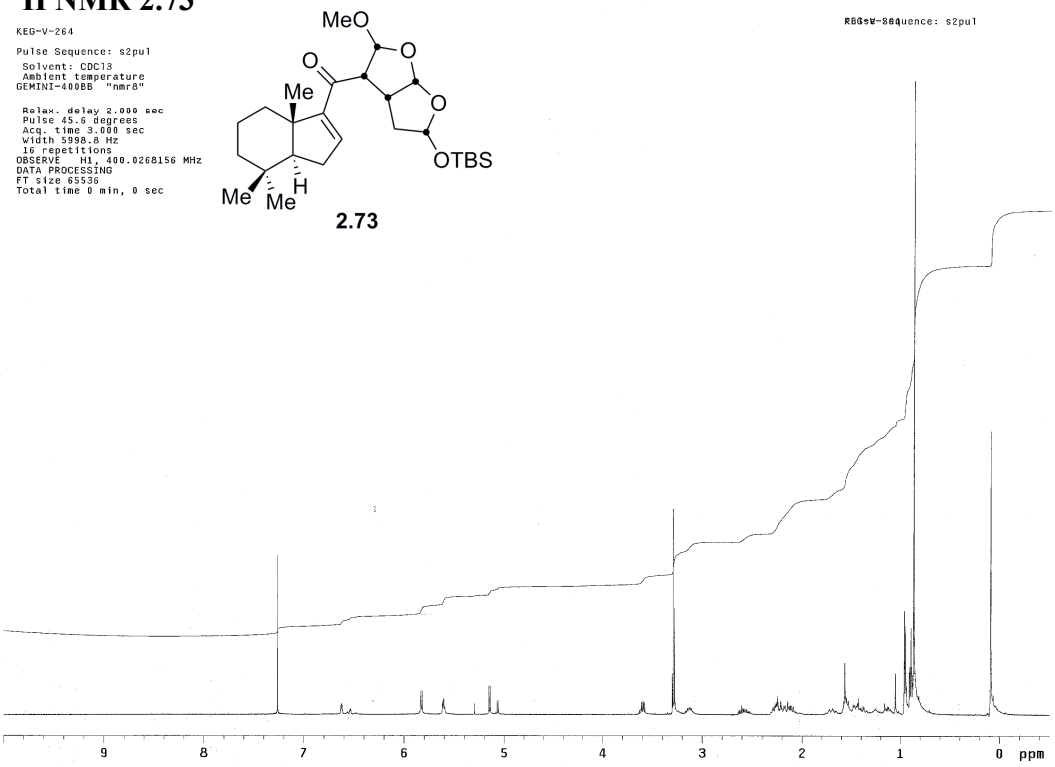
2.72

^{13}C NMR 2.72

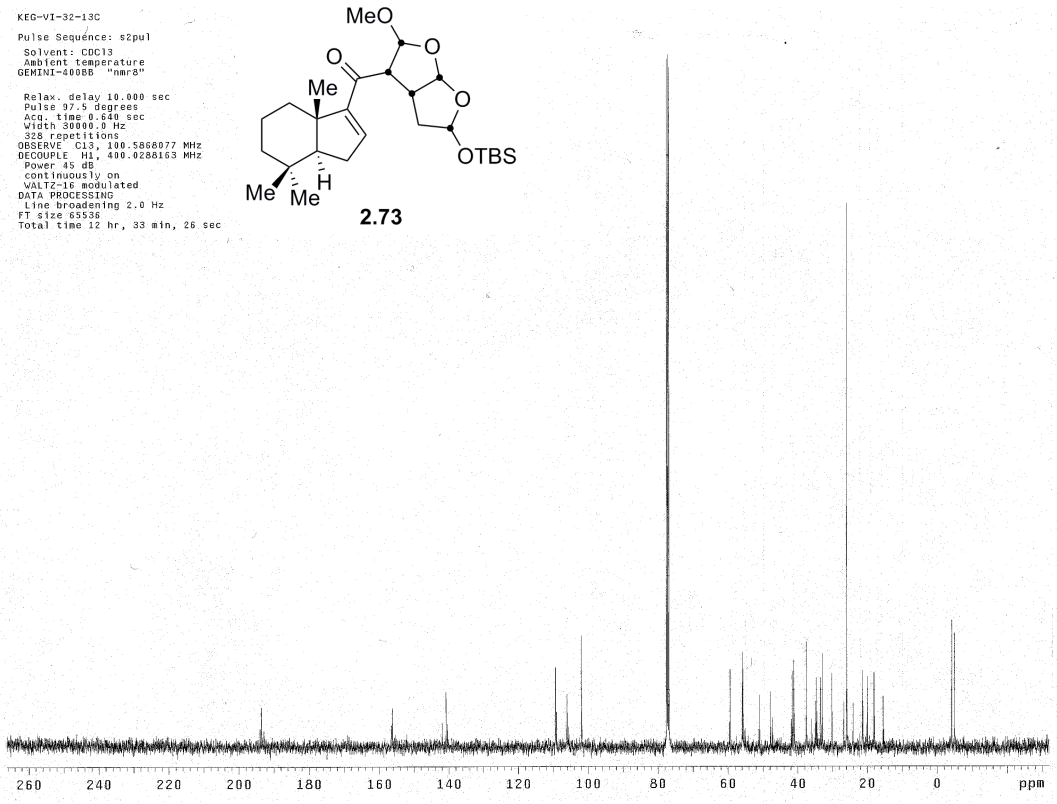


2.72

^{1}H NMR 2.73



^{13}C NMR 2.73

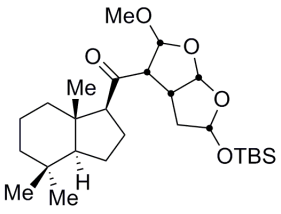


^{1}H NMR 2.74

KEG-V-221 purified

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 45.6 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
16 repetitions
OBSERVE H1, 400.0268160 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



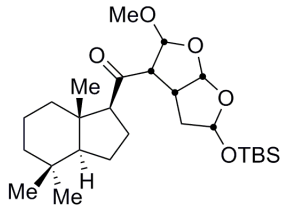
2.74

^{13}C NMR 2.74

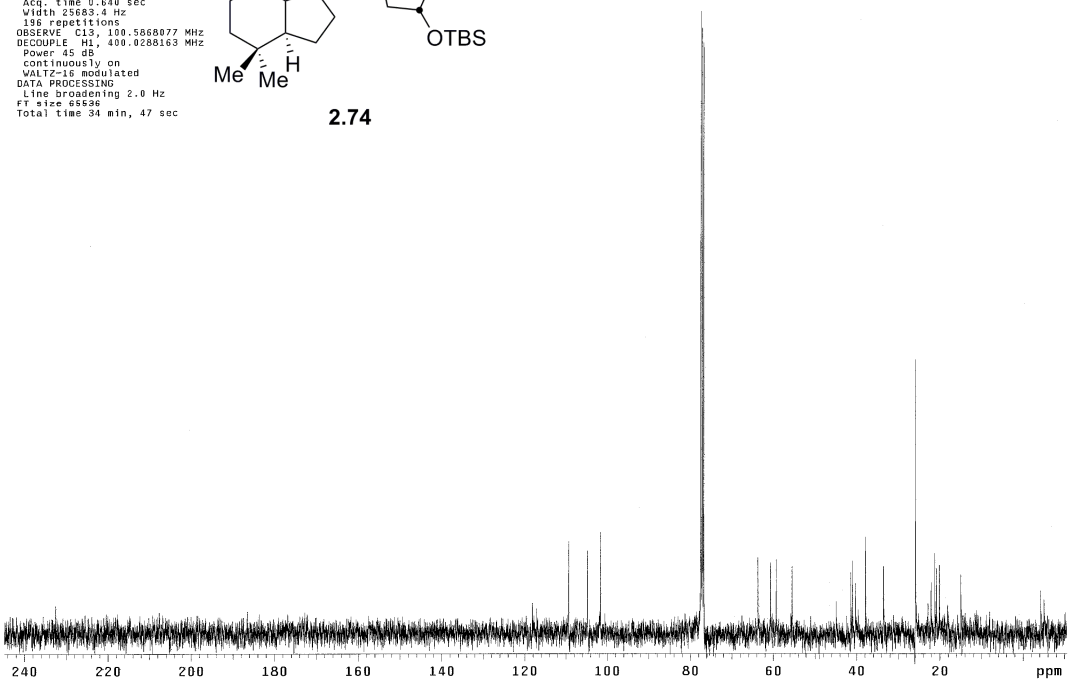
13C OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 4.000 sec
Pulse 81.2 degrees
Acq. time 0.640 sec
Width 25683.4 Hz
196 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 34 min, 47 sec



2.74

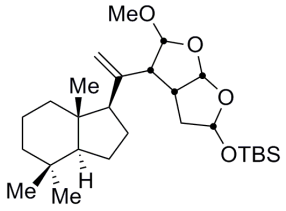


^{1}H NMR 2.83

KEG-V-270

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 42.3 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
8 repetitions
OBSERVE H1, 400.0268150 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



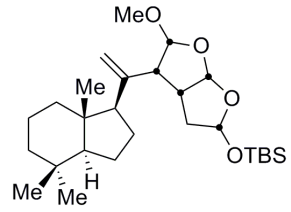
2.83

^{13}C NMR 2.83

KEG-VI-61 13C

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 6.000 sec
Pulse 97.5 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
2032 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 16 hr, 13 min, 33 sec



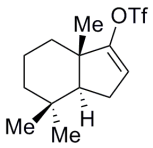
2.83

^{1}H NMR 2.84

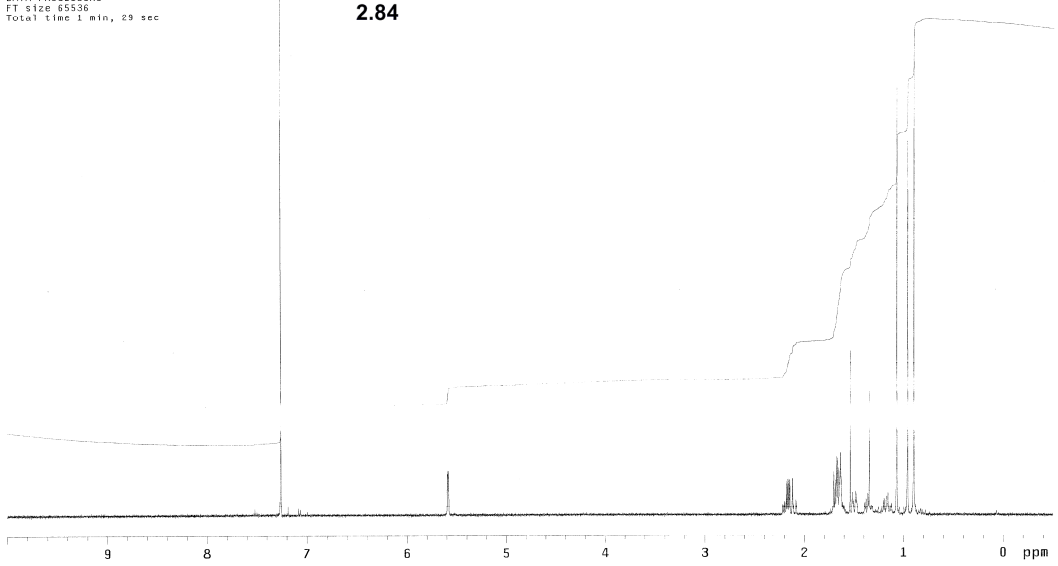
KEG-VI-94

Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 40.4 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
12 repetitions
OBSERVE H1, 400.0268158 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



2.84

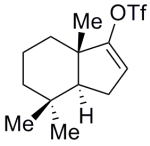


^{13}C NMR 2.84

KEG-VI-94-13C

Solvent: CDCl3
Ambient temperature
File: KEG-VI-94-13C
GEMINI-400BB "nmr8"

Relax. delay 4.000 sec
Pulse 64.8 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
8520 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 29 hr, 27 min, 13 sec



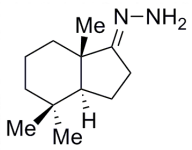
2.84

^{1}H NMR 2.85

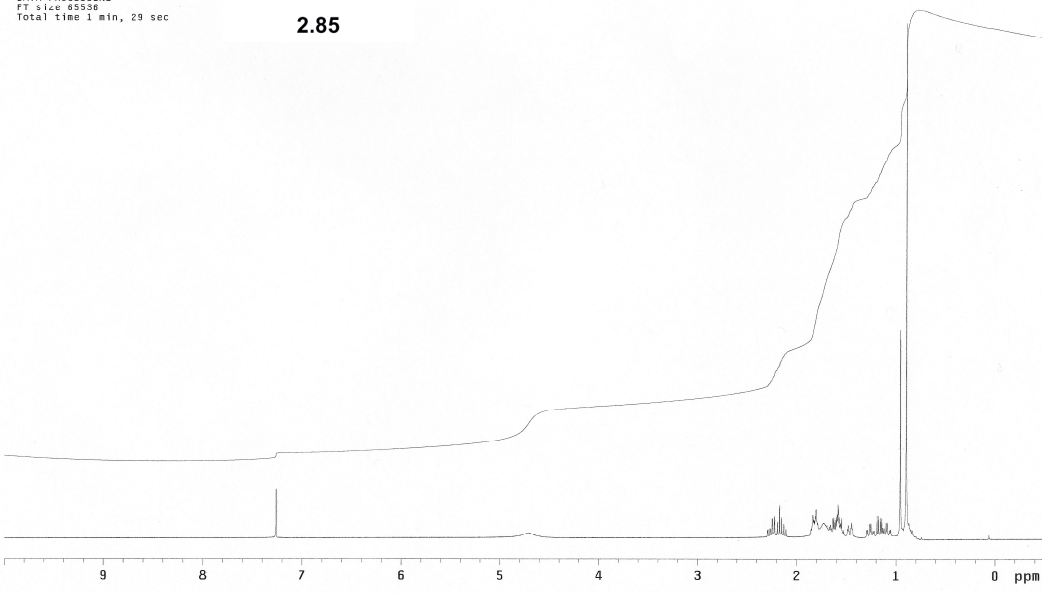
KEG-VI-17

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 42.3 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
16 repetitions
OBSERVE H1, 400.0268147 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



2.85

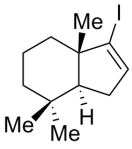


^{1}H NMR 2.86

KEG-VI-18

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 42.3 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
8 repetitions
OBSERVE H1, 400.0268154 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



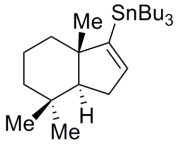
2.86

^{1}H NMR 2.88

KEG-VI-72

Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 42.3 degrees
Acq. time 3.000 sec
Width 5998.8 Hz
12 repetitions
OBSERVE H1, 400.0268158 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



2.88

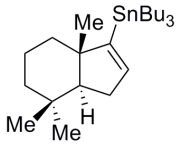
^{13}C NMR 2.88

KEG-VI-72 13C

KEG-VI-72 13C

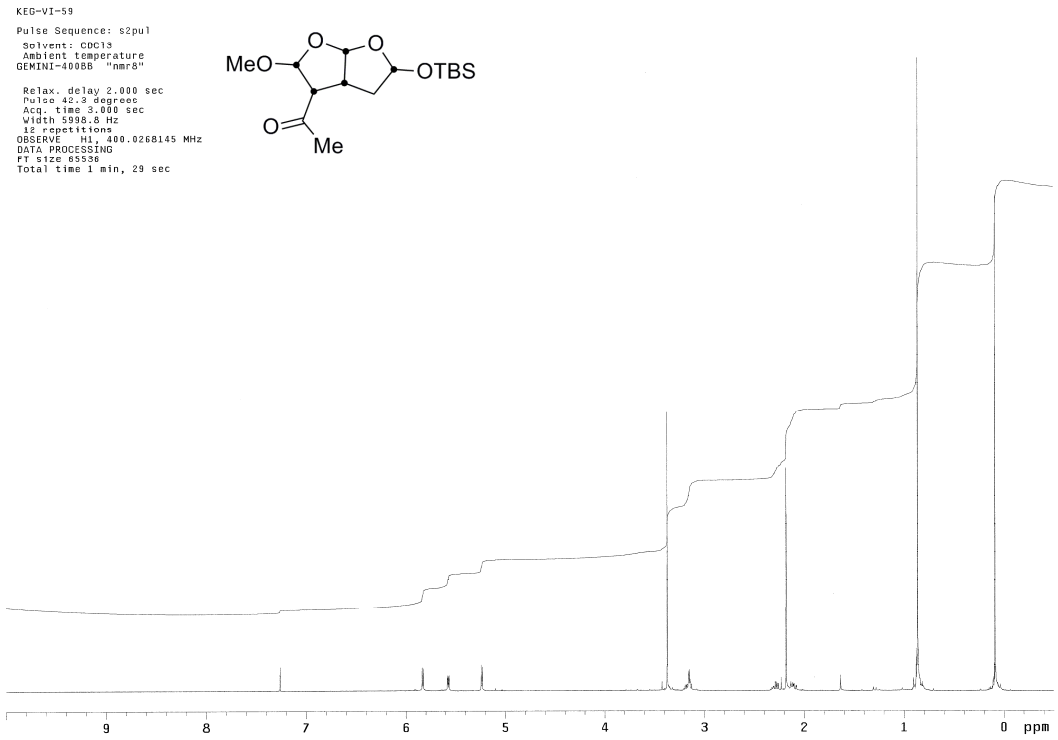
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 10.000 sec
Pulse 97.5 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
388 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 12 hr, 33 min, 26 sec

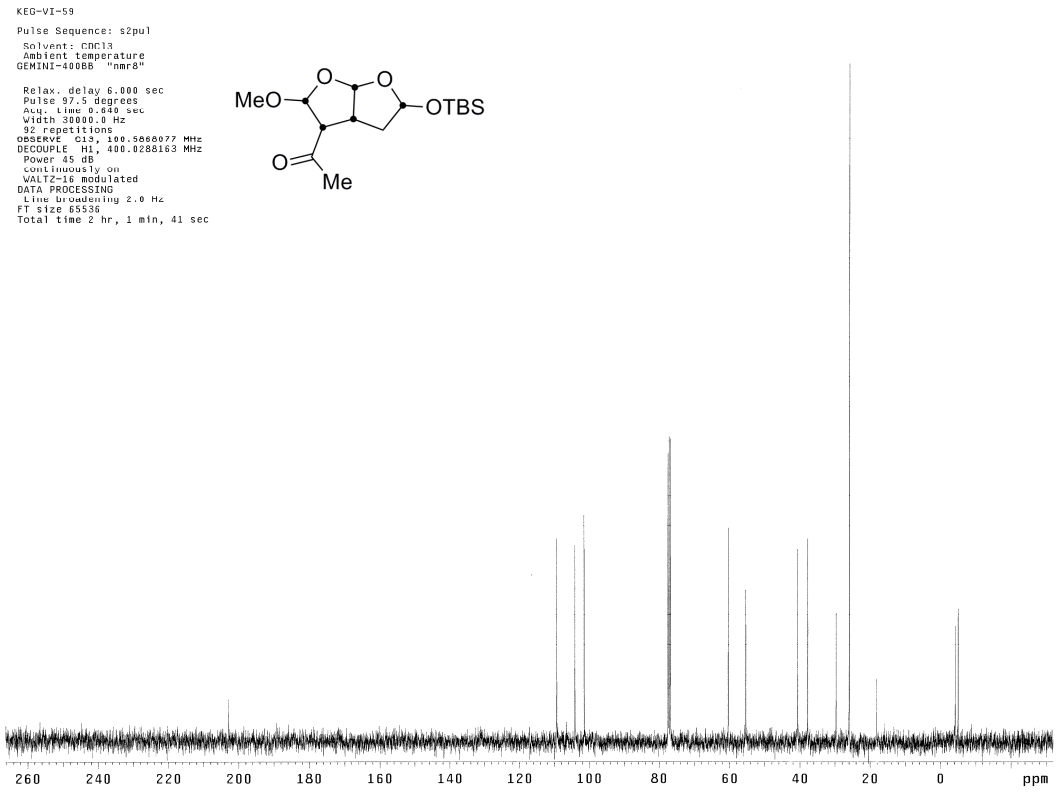


2.88

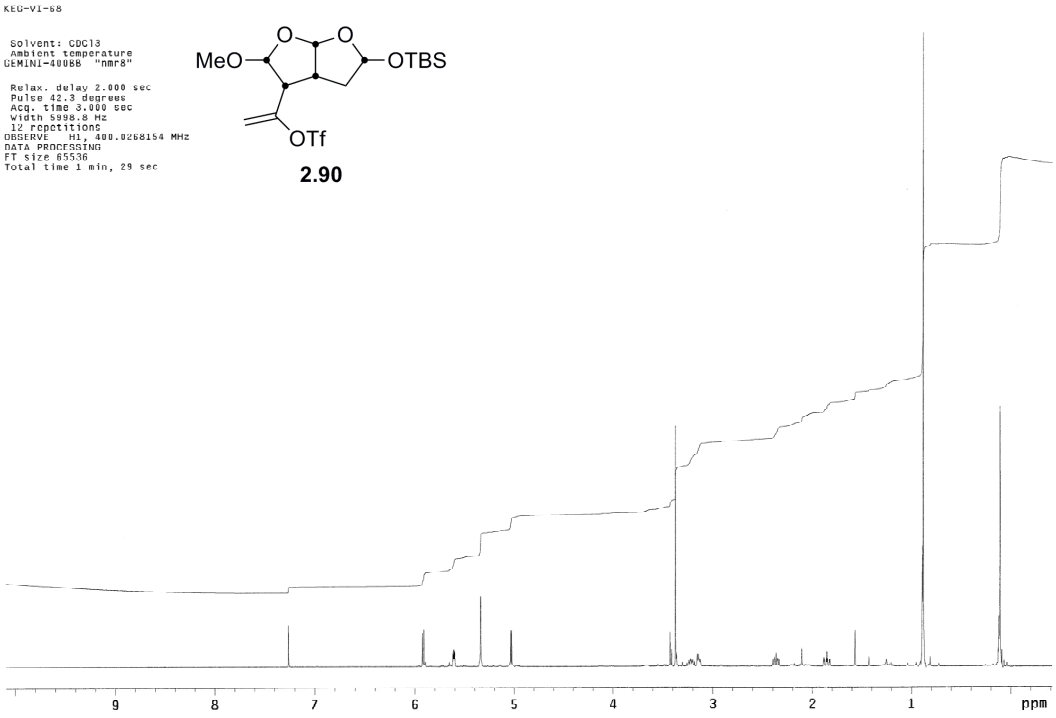
^{1}H NMR Methyl Ketone



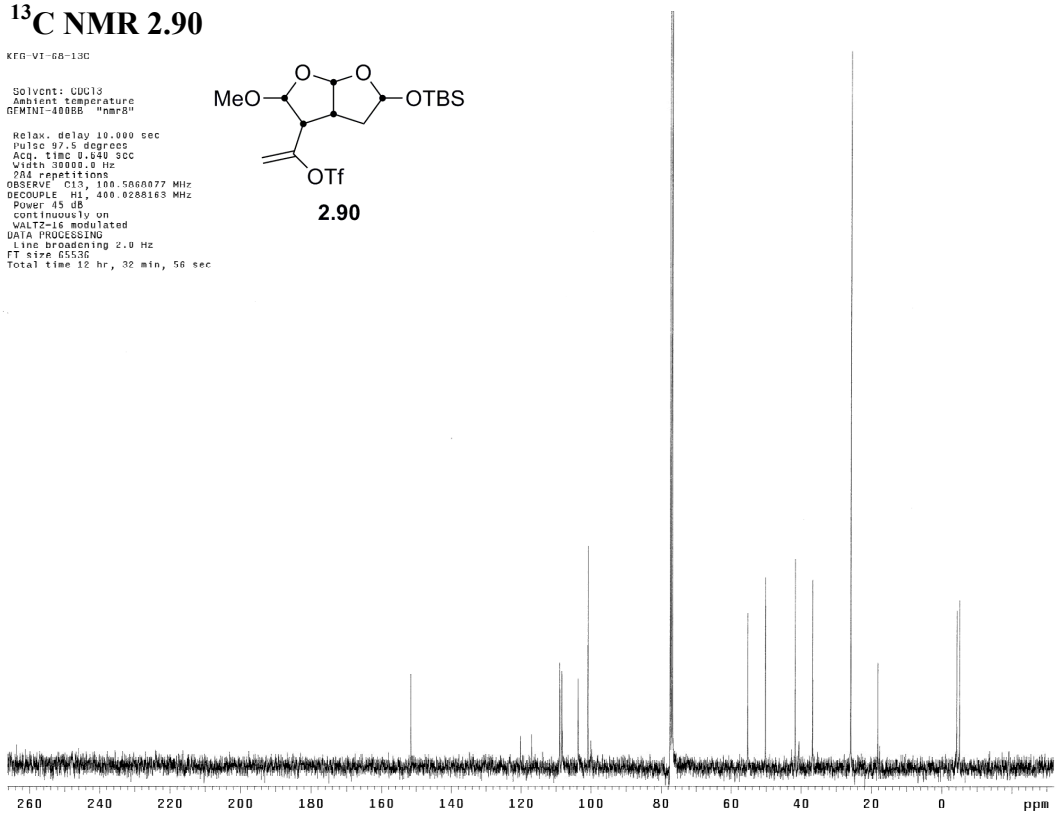
^{13}C NMR Methyl Ketone



^{1}H NMR 2.90



^{13}C NMR 2.90

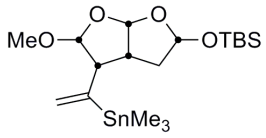


^{1}H NMR 2.91

KEG-VI-89 fractions 21-27

Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 2.000 sec
Pulse 40.4 degrees
Acq. time 3.000 sec
Width 5398.8 Hz
12 repetitions
OBSERVE H1, 400.0268156 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 29 sec



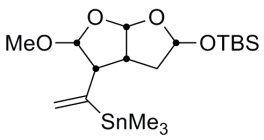
2.91

^{13}C NMR 2.91

KEG-VI-89 13C

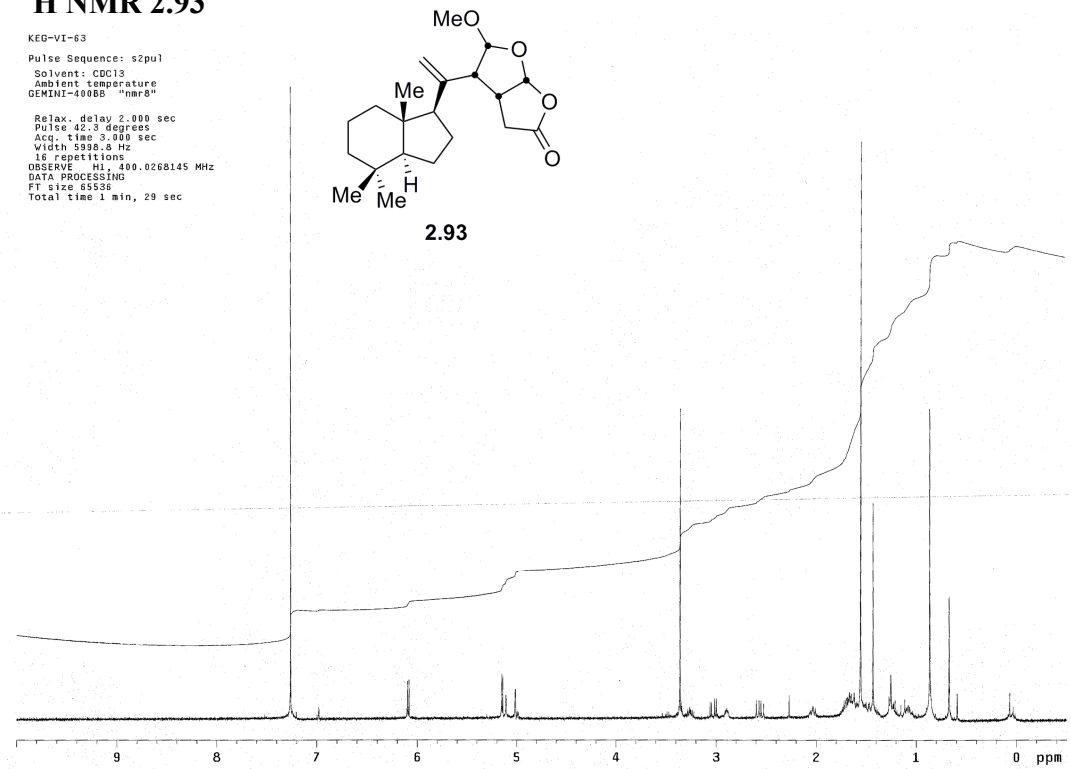
Solvent: CDCl3
Ambient temperature
GEMINI-400BB "nmr8"

Relax. delay 4.000 sec
Pulse 64.8 degrees
Acq. time 0.640 sec
Width 30000.0 Hz
640 repetitions
OBSERVE C13, 100.5868077 MHz
DECOUPLE H1, 400.0288163 MHz
Power 45 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 2 hr, 56 min, 43 sec

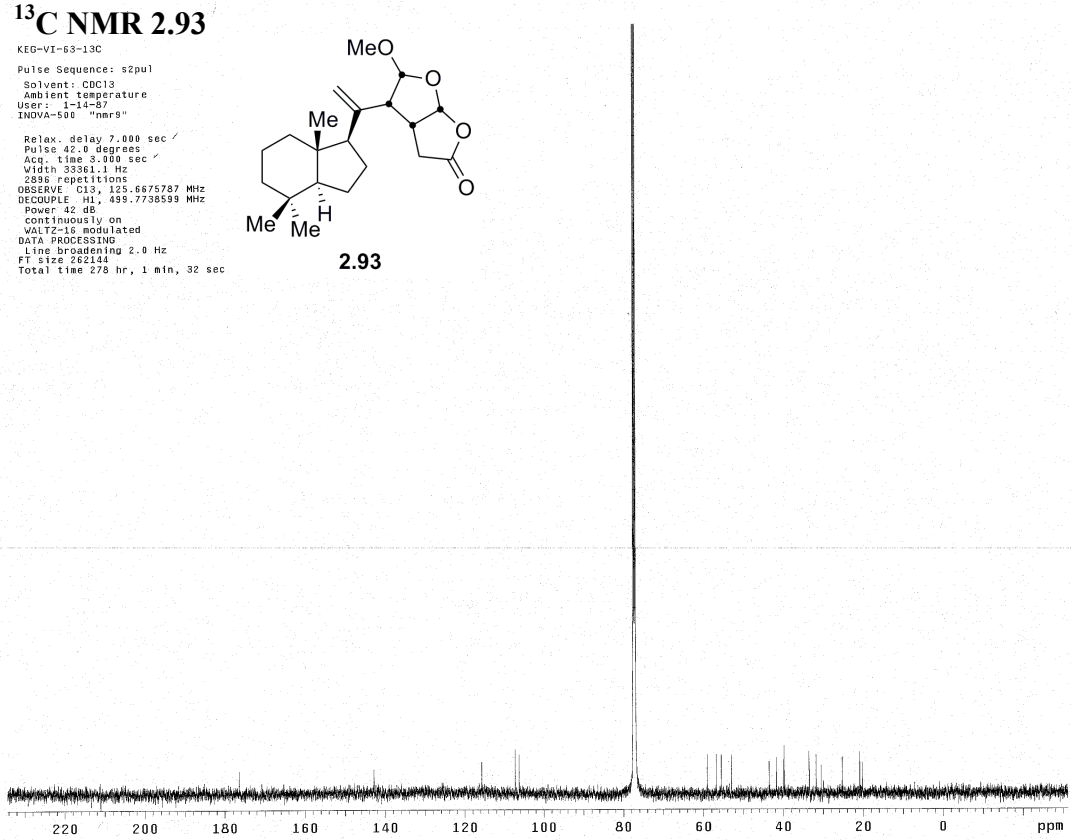


2.91

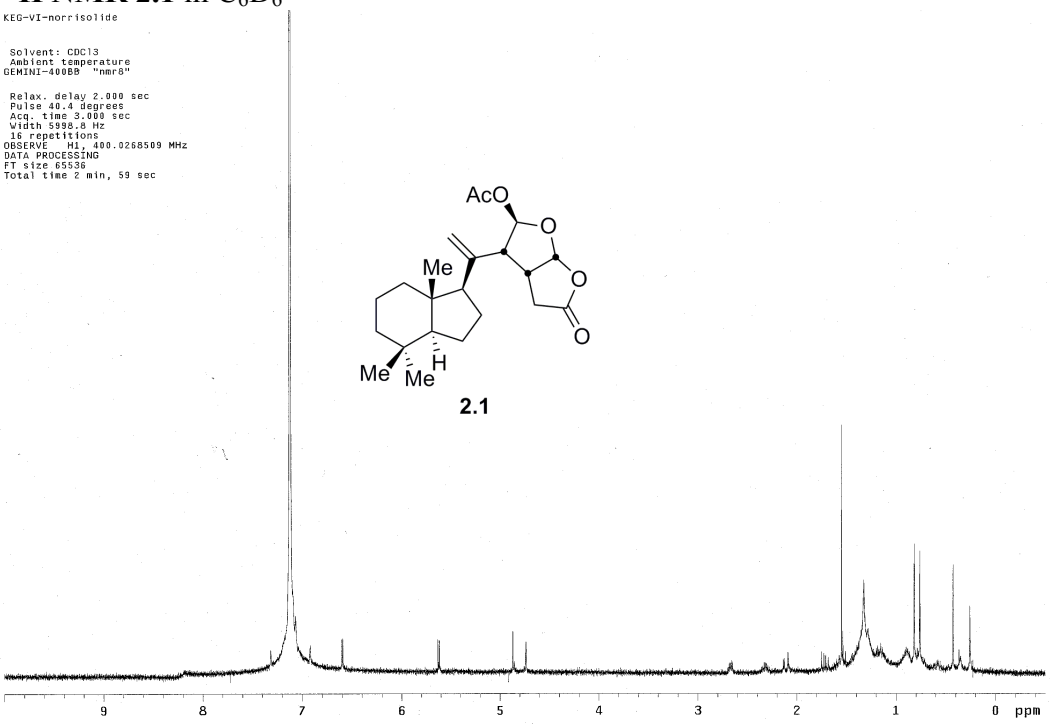
^{1}H NMR 2.93



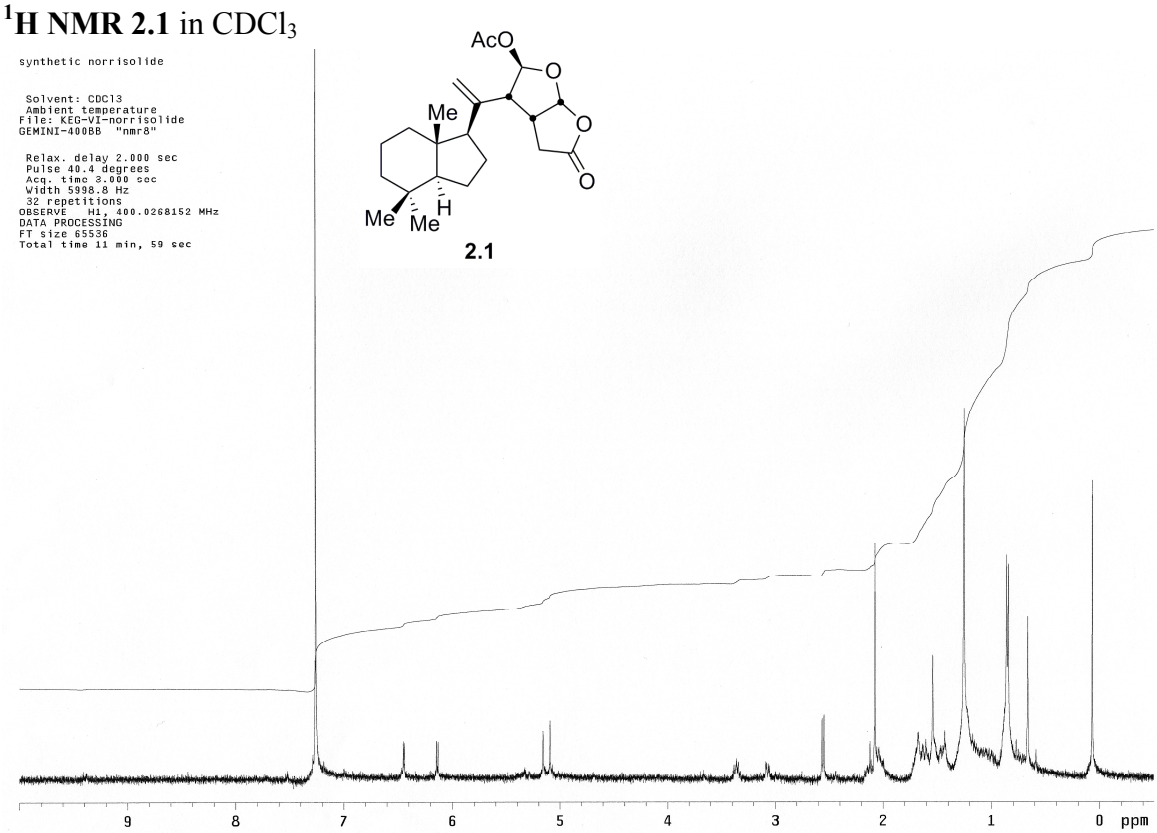
^{13}C NMR 2.93



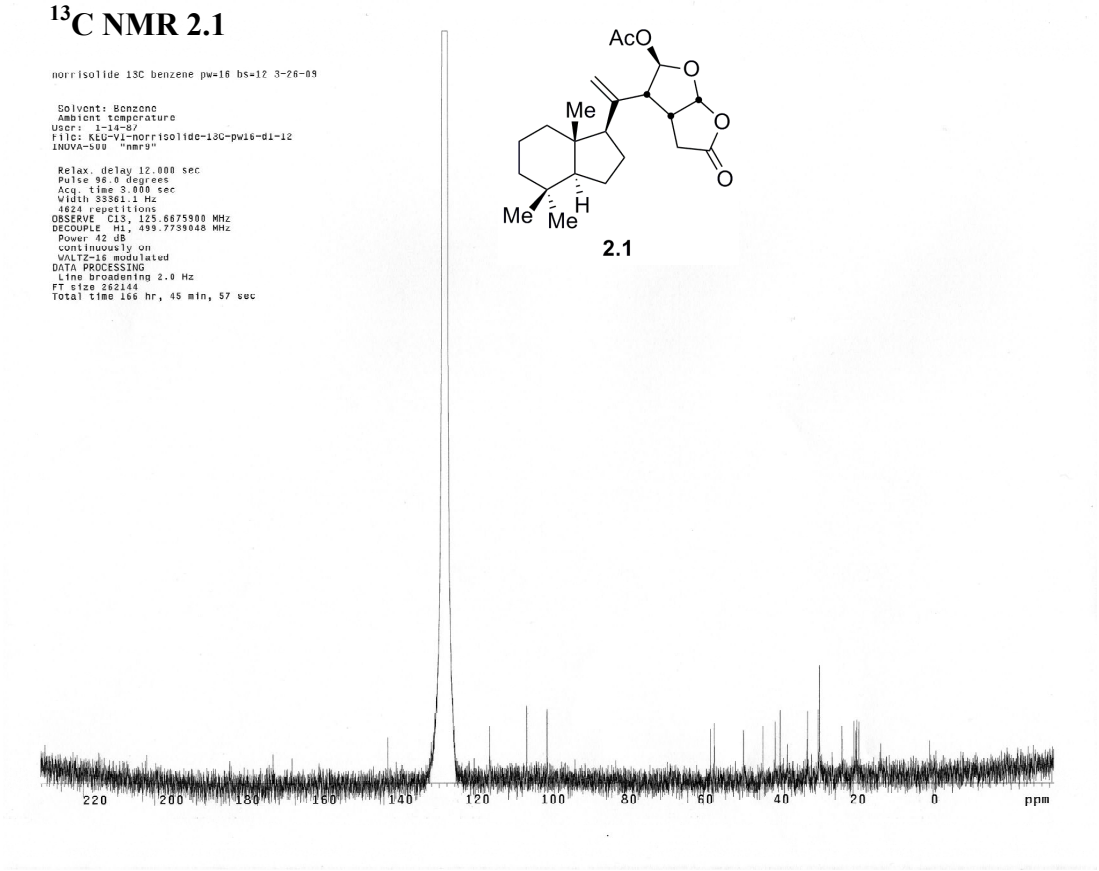
^{1}H NMR 2.1 in C_6D_6



^{1}H NMR 2.1 in $CDCl_3$



^{13}C NMR 2.1

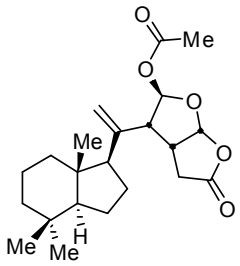

norrisolide 13C benzene pw=16 bs=12 3-26-09
Solvent: Benzene
Ambient temperature
User: 1-14-87
File: KEU-Vi-norrisolide-13C-pw16-d1-12
INOVA-500 "nmr9"
Relax. delay 12.000 sec
Pulse 96.0 degrees
Acq. time 3.000 sec
Width 33361.1 Hz
4624 repetitions
OBSERVE C13, 125.6675900 MHz
DECOUPLE H1, 499.7739048 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 262144
Total time 166 hr, 45 min, 57 sec
AcO
O
Me
O
O
Me
Me
H
2.1
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Chapter 3

Cellular Interactions of Norrisolide:

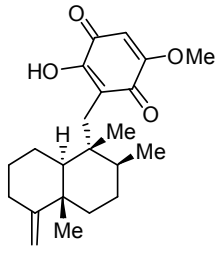
Preliminary Investigations

3.1 Introduction



3.1

As discussed in the previous chapter, our initial interest in norrisolide (**3.1**) was garnered by the reports that it induced irreversible Golgi fragmentation.[1] The Golgi apparatus is an organelle that plays a pivotal role in processing, sorting and transporting proteins within the cell.[2] However, for all of the information that is currently known about the Golgi, there are still details of organization and function that are unknown. Uncovering specific targets of natural products that disrupt the functioning of the Golgi is one way to further understand this complex organelle.



3.2

In addition to norrisolide, there are many natural products that induce Golgi disruption. One such compound is ilimaquinone (**3.2**), which has been studied in our laboratories.[3] It was found that

[1] Kim, C.; Hoang, R.; Theodorakis, E. A. *Org. Lett.* **1999**, *1*, 1295-1297.
[2] For an overview of Golgi function, see: a) Jamieson, J. D. *Biochim. Biophys. Acta,* **1998**, *1404*, 3-8. b) *The Golgi Apparatus* Mirinov, A.; Pavelka, M., Eds.; SpringerWien: NewYork, 2008.
[3] a)Bruner, S. D.; Radeke, H. S.; Tallarico, J. A.; Snapper, M. L. *J. Org. Chem.* **1995**, *60*, 1114-1115. b) Radeke, H. S.; Digits, C. A.; Bruner, S. D.; Snapper, M. L. *J. Org. Chem.* **1997**, *62*, 2823-2831. c) Radeke, H. S.; Snapper, M. L. *Bioorg. Med. Chem. Lett.* **1998**, *6*, 1227-1232. d) Radeke, H. S.; Digits, C. A.;

ilimaquinone inhibits *S*-adenosylhomocysteine hydrolase, which blocks new methylations in the cell. The link between reduced methylations and the disruption of the Golgi apparatus was postulated to be through the Rab6 protein, which has been reported to function in the transport of vesicles from the nucleus of the cell to the Golgi, and also from the Golgi to the endoplasmic reticulum (ER). Rab6 is methylated by *S*-adenosylmethionine. Methylation is a posttranslational modification that makes proteins more hydrophobic, making it easier to bind with membranes or other proteins. If new methylations are blocked, Rab6 would remain in the cytoplasm, along with the transport vesicles. The effect of ilimaquinone can be reversed through the addition of excess *S*-adenosylmethionine, which allows methylations to resume.

Other compounds that have been reported to induce Golgi disruption include brefeldin A, monensin, bafilomycin, retinoic acid, okadaic acid and nocodazole (Figure 3.1).[4]

Brefeldin A (**3.3**) changes the morphology of the Golgi by fusing the cisternae, the flat stacks that make up the Golgi apparatus. In addition to fusing the cisternae, brefeldin A also induces tubule formation between the Golgi and the ER by inhibition of guanine-nucleotide exchange factors (GEF). GEF is an accessory protein that catalyzes the transformation of GDP to GTP. GEF is bound to ADP-ribosylation factor 5 (ARF5), the function of which is protein transport through the ER-Golgi systems[5] ARF5 interacts weakly with membranes when bound to GDP; this interaction becomes strong when

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Casaubon, R. L.; Snapper, M. L. *Chem. Biol.* **1999**, *6*, 639-647. e) Casaubon, R. L.; Snapper, M. L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 133-136.

[4] For a review on Golgi-disturbing agents, see: Dinter, A.; Berger, E. G. *Histochem. Cell Biol.* **1998**, *109*, 571-590.

[5] Jackson, C. L.; Casanovoa, J. E. *Trends Cell. Biol.* **2000**, *10*, 60-67.

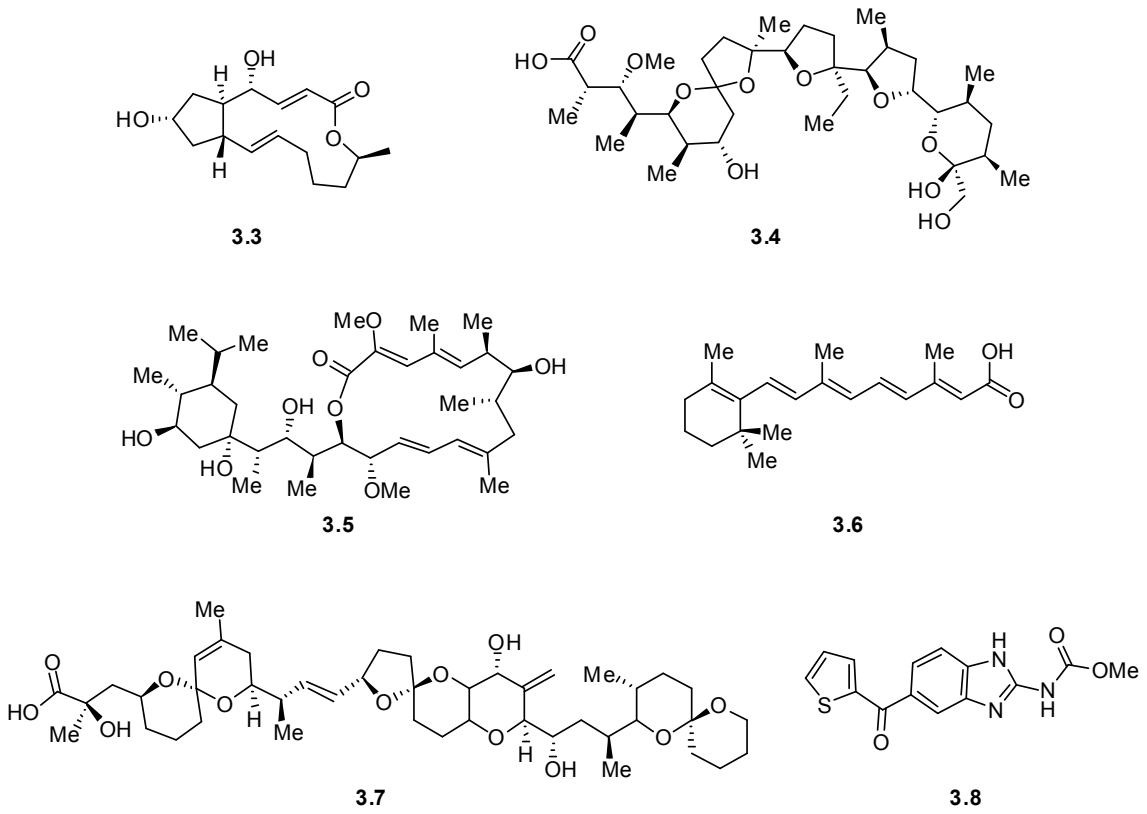

Figure 3.1 Golgi-disrupting natural products.

bound to GTP. By inhibiting a domain common to all GEF proteins, brefeldin A shuts down Golgi-membrane activity. These effects on the Golgi complex are completely reversible within 120 minutes after removal of brefeldin A from the cell.[6]

Monensin (**3.4**) affects the Golgi complex by inducing swelling that increases from the *cis* side to the *trans* side of the Golgi. This swelling affects a number of Golgi functions, such as peripheral glycolsylation due to slowed trafficking within the Golgi. The direct mode of action is not clear, although breakdown of acidification mechanisms within the cell are thought to be related.

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[6] Strous, G. J.; Berger, E. G.; van Kerkhof, P.; Bosshart, H.; Berger, B.; Geuze, H. J. *Biol. Cell.* **1991**, *71*, 25-31.

Bafilomycin (**3.5**) also induces swelling in the Golgi complex, but to a much lesser extent than monensin. Bafilomycin inhibits vacuolar ATPases, one of which is found in the Golgi apparatus. This particular ATPase is responsible for acidifying the interior of the Golgi to a pH of 6.5, potentially interfering with proton translocating activity.

Retinoic acid (**3.6**) induces a reversible change on the Golgi, which varies slightly depending on the cell line tested. General effects include disappearance of cisternae and the appearance of vacuoles. The mechanism of action of retinoic acid has not yet been elucidated; however, it has been postulated to interact with protein kinase C pathways. Protein kinase C is involved in regulating membranous organelles.

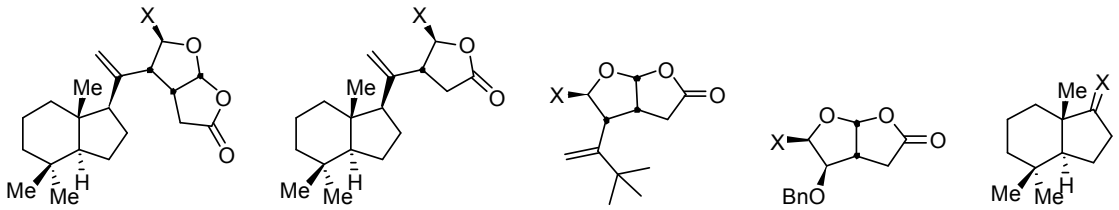
Introduction of okadaic acid (**3.7**) into cells collapses the Golgi complex into a cluster of tubules and vesicles in a process that begins with swelling of the cisternae, followed by vesiculation. It was also found that compounds in the Golgi were transferred to the ER. These effects are reversible, although the amount of reversibility is dependent on the amount of time okadaic acid was incubated with the cell.

Nocodazole (**3.8**) has been shown to depolymerize microtubules in an energy-dependent process, which causes a breakdown in the structure of the Golgi apparatus and disrupts transport from the ER to the Golgi. Within 90 minutes of introducing nocodazole into the cell the Golgi becomes vesiculated. This effect is reversible within 30 minutes of nocodazole being removed from the cell.

3.2 Previous Biological Studies with Norrisolide

The Theodorakis group, after completing the total synthesis of norrisolide, focused their efforts on identifying norrisolide's biological target.[7] An initial report in 2004 looked at a variety of analogues (Figure 3.2) and determined which portions of the natural product were necessary for activity.[8] Analogues tested included methyl ketone **3.9**, truncated analogues **3.10** and **3.11**, *t*-butyl analogues **3.12** and **3.13**, norrisane fragments **3.14** and **3.15**, and hydrindane fragments **3.16** and **3.17**.

These studies reinforced that in normal rat kidney (NRK) cells norrisolide produces an irreversible vesiculation of the Golgi apparatus at a concentration of 30 μM at 37 °C. Compounds **3.9**, **3.10** and **3.11** showed reversible fragmentation; the Golgi apparatus reformed when the compounds were washed out of the cells with buffer. Compounds lacking the hydrindane portion of the molecule (**3.12**, **3.13**, **3.14** and **3.15**) showed no vesiculation of the Golgi. Introduction of **3.16** or **3.17** into the cell also failed to induce Golgi fragmentation. These results lead to two conclusions. First, the acetate acetal is necessary to obtain irreversible Golgi fragmentation. Secondly, the hydrindane



3.1 X=OAc
3.9 X=Ac

3.10 X=OAc
3.11 X=Ac

3.12 X=OAc
3.13 X=Ac

3.14 X=OAc
3.15 X=Ac

3.16 X=O
3.17 X=CH_2

Figure 3.2 Norrisolide analogues in Theodorakis' initial biological studies.

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[7] Brady, T. P.; Kim, S. H.; Wen, K.; Theodorakis, E. A. *Agnew. Chem. Int. Ed.* **2004**, *43*, 739-742.
[8] Brady, T. P.; Wallace, E. K.; Kim. S. H.; Guizzunti, G.; Malhotra, V.; Theodorakis, E. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5035-5039.

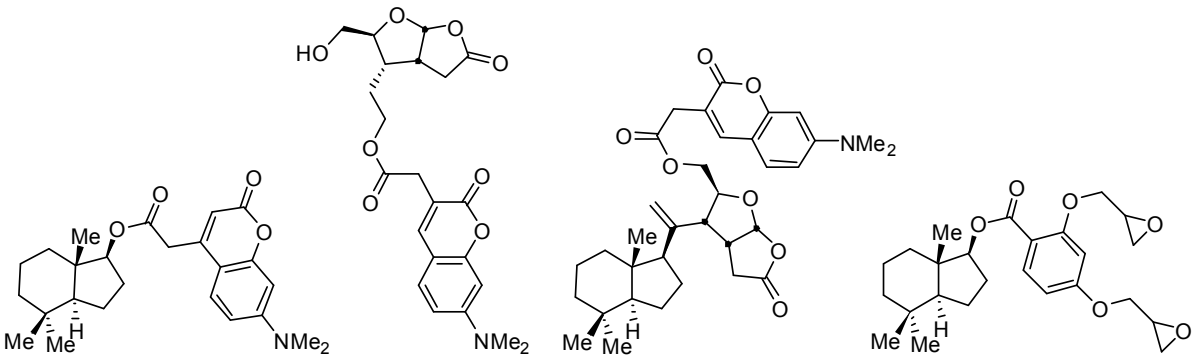


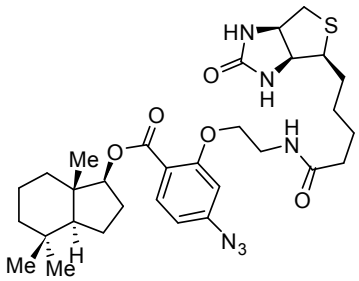
Figure 3.3 More advanced analogues of norrisolide from the Theodorakis group.

core must be bound to the norrisane side chain for there to be any activity. It is postulated that the hydrindane core is needed for binding and that the acetate group is essential for irreversible vesiculation.

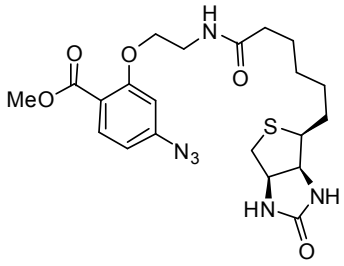
Following these results, the Theodorakis group made a series of fluorescent probes, **3.18-3.20** (Figure 3.3) to see where the compounds were localized in the cell.[9] As expected, analogue **3.19** with no hydrindane core had no effect on the Golgi apparatus. Analogues **3.18** and **3.20** both reversibly vesiculated the Golgi in NRK cells and were found to localize on the Golgi apparatus. To reinforce the concept that a side chain with an electrophilic moiety needs to be attached to the hydrindane core in order to produce an irreversible effect, **3.21** was synthesized and shown to induce irreversible Golgi fragmentation.

With a firmer understanding of the requirements for irreversible Golgi fragmentation, the Theodorakis group turned their attention to trying to isolate the

[9] Guizzunti, G.; Brady, T. P.; Malhotra, V.; Theodorakis, E. A. *J. Am. Chem. Soc.* **2006**, *128*, 4190-4191.



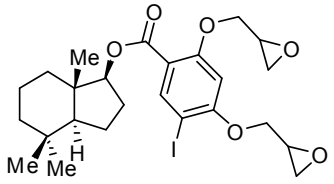
3.22



3.23

Figure 3.4 Biotin compounds with aryl azides synthesized by Theodorakis.

biological target of norrisolide.[10] Biotin analogue **3.22** was synthesized, along with **3.23**, which lacks the hydrindane core (Figure 3.4). After incubation with NRK cells and UV irradiation, it was found that both **3.22** and **3.23** bound to the same proteins. Without a unique interaction between **3.22** and a protein, the biological target of norrisolide could not be isolated. Similar studies were done replacing the azide with epoxides, like those found in analogue **3.21**. However, these compounds displayed less than ten percent vesiculation of the Golgi apparatus, which was reversible. The length of the biotin tether may be playing a role in the decreased activity of this analogue. To eliminate any interference from the biotin, a radiolabeled analogue has been planned. Incorporation of iodine into compound **3.21** gives **3.24**, which performs comparably to **3.21**, giving irreversible Golgi fragmentation. Installation of ^{125}I would give a handle with which to isolate the biological target of norrisolide.



3.24

[10] Guizzunti, G.; Brady, T. P.; Malhotra, V.; Theodorakis, E. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 320-325.

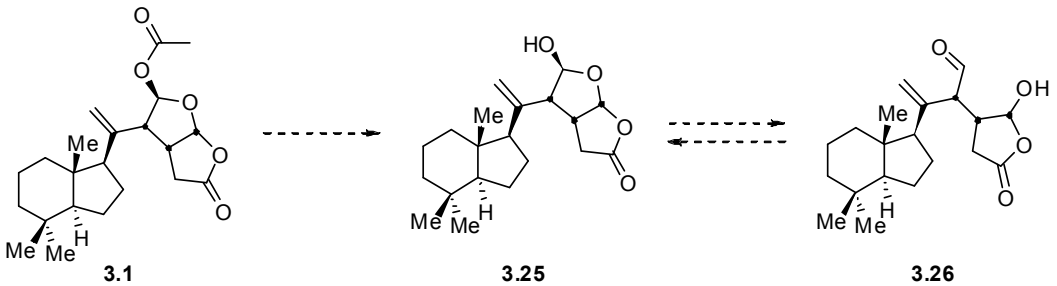
3.3 Our Approach to Isolating the Biological Target of Norrisolide

With the data from the Theodorakis group in hand, we came to the hypothesis that binding of norrisolide to its biological target could have two possible modes of action. First, norrisolide could be acting as an acyl transfer reagent, explaining why the acetate group is necessary for reactivity. Secondly, it is possible to think of the acetate acetal as a masked aldehyde (Scheme 3.1). If this aldehyde is unmasked, norrisolide could form a Schiff base with a lysine residue. It appears unlikely that interaction with the biological target could be taking place through the lactone, since many of Theodorakis' substrates which contain the lactone component and do not have activity comparable to norrisolide. A third possibility is the elimination of the acetate group to form an oxonium ion and addition of a nucleophilic moiety.

If the second mode of action described above is operational the Schiff base could in turn be reduced with ^{3}[H]-$NaBH_4$ to form a radiolabeled norrisolide-protein complex which could then be isolated and characterized.

Reductive alkylation of proteins is a well known technique that has been reviewed in the literature.[11] Reductive methylation with formaldehyde is the most prominent in the

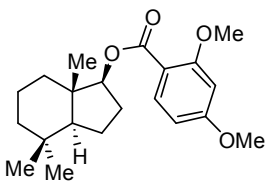
Scheme 3.1 Possible transformation of norrisolide to an aldehyde.



literature, but higher aldehydes, such as glyceraldehyde, glucose, fructose or lactose have also been described.[12,13] In addition, reports of tritium labeling of proteins using this technique are also well known.[14] Using these techniques, it was postulated that isolation of a radiolabeled norrisolide-protein complex may be possible.

3.4 Reductive Alkylation Labeling Study

Norrisolide and control compound **3.27**, known from Theodorakis' studies to produce reversible Golgi fragmentation, were the main compounds of interest for our reductive alkylation experiments. Bovine liver extract was incubated with norrisolide, **3.27**, a mixture of norrisolide and **3.27** (to see if there is a competition effect), and with no compound. Each reaction was reduced with ^{3}[H]-$NaBH_4$ and initially purified by ion exchange chromatography.



3.27

As seen in Chart 3.1, the majority of radiolabeled proteins were liberated from the ion exchange resin during the wash with the initial zero-salt buffer. This was confirmed by SDS-PAGE; only the first fraction from each reaction contained appreciable amounts of protein. The first fraction of each reaction was further purified via size exclusion chromatography, with similar results. Again, there was minimal separation of labeled proteins, as shown in Chart 3.2.

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[11] Means, G. E.; Feeney, R. E. *Analytical Biochemistry*, **1995**, *224*, 1-16.
[12] Acharya, A. S.; Mannin, J. M. *J. Biol. Chem.* **1980**, *255*, 1406-1412.
[13] Lee, H. S.; Sen, L. C.; Clifford, A. J.; Whitaker, J. R.; Feeney, R. E. *J. Agric. Food. Chem.* **1979**, *27*, 1094-1098.
[14] Tack, B. F.; Cean, J.; Eilat, D.; Lorenz, P. E.; Scheechter, A. N. *J. Biol. Chem.* **1980**, *255*, 8842-8847.

Each fraction displaying radioactivity from the size exclusion column was separately concentrated and run on SDS-PAGE. The gel separated five protein bands which were identical for both the blank and the reaction containing norrisolide. Figure

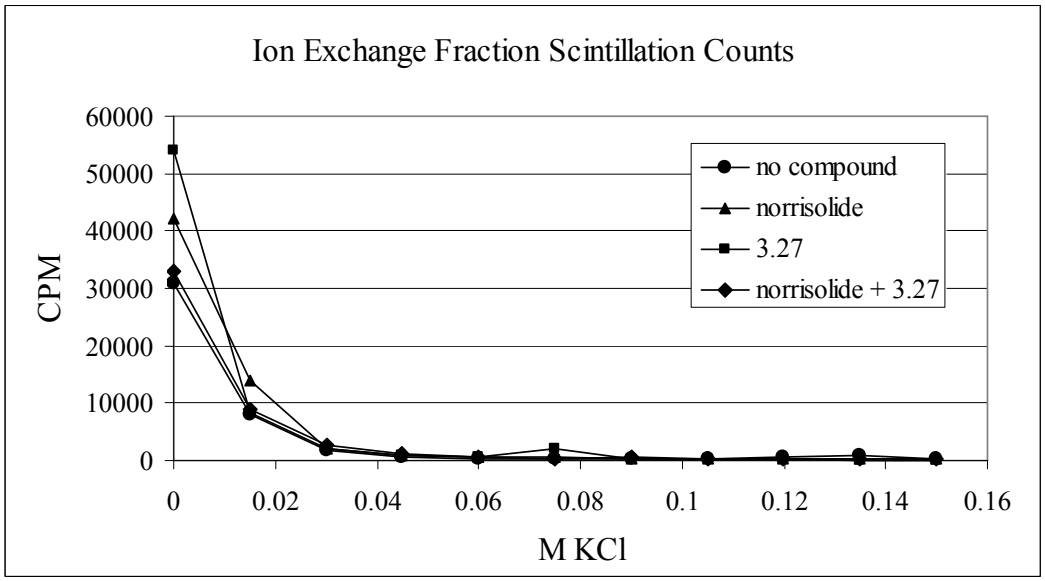


Chart 3.1 Scintillation count of the fractions from ion exchange chromatography.

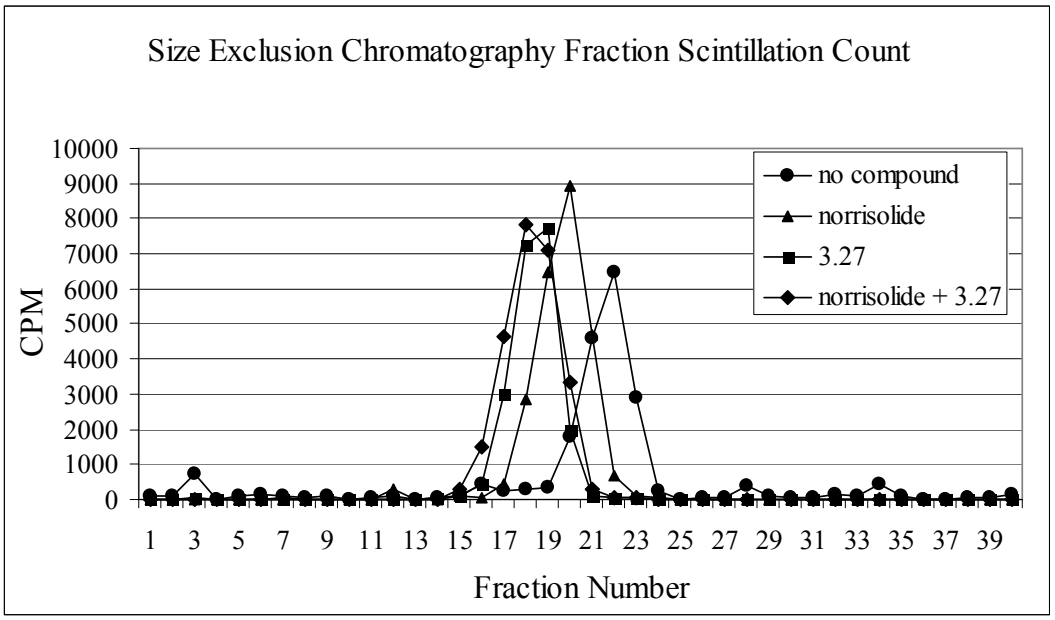


Chart 3.2 Scintillation count of the fractions from size exclusion chromatography.

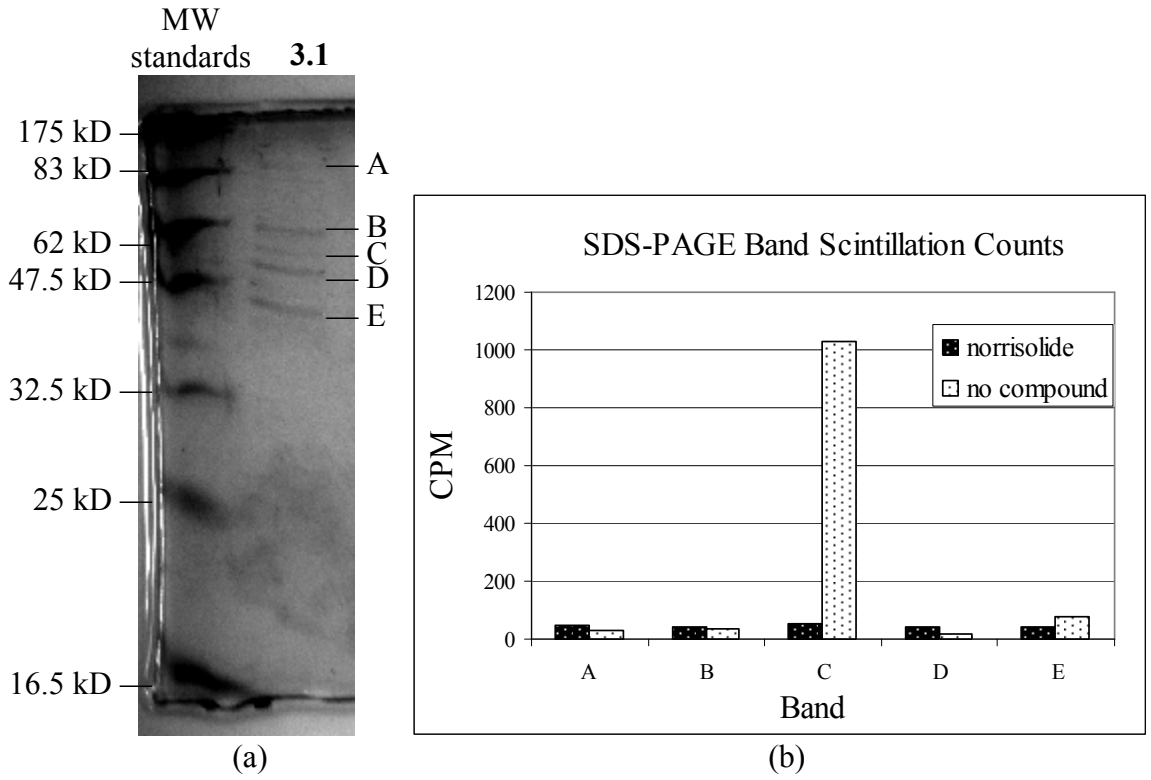
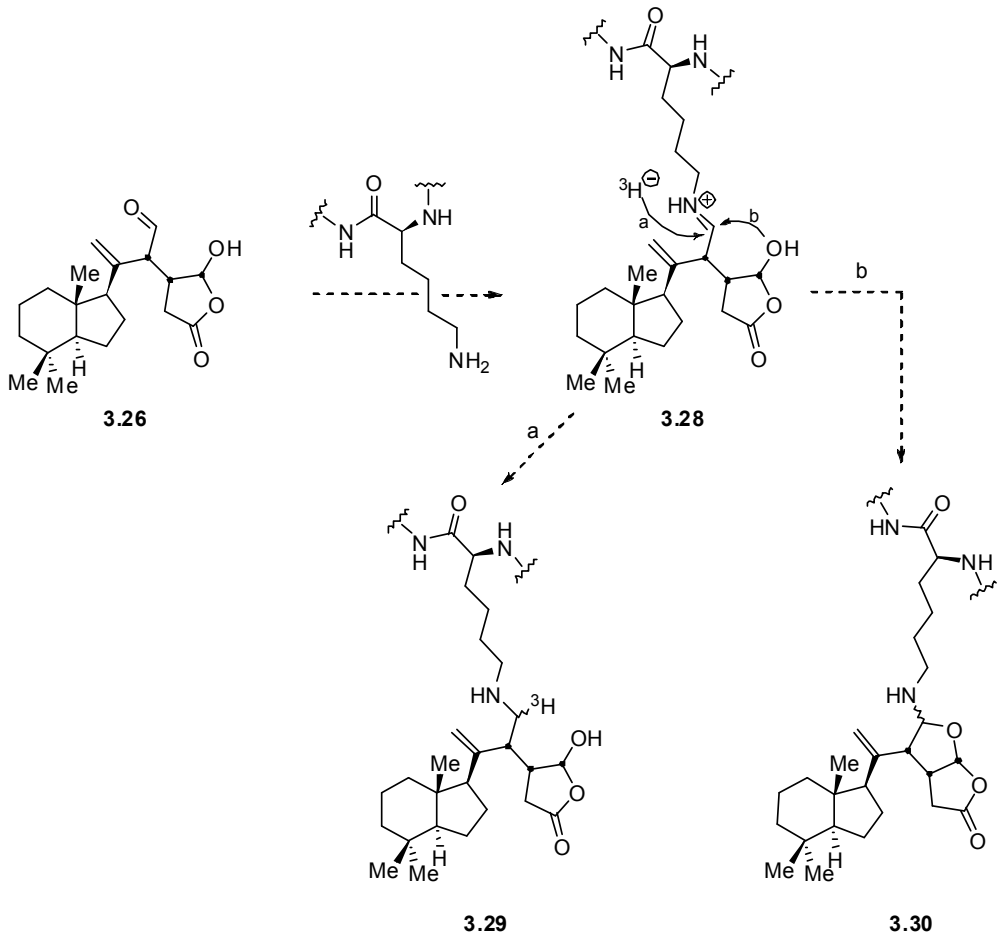


Figure 3.5 (a) SDS-PAGE gel of the reaction with norrisolide (right) and molecular weight standards (left). (b) Comparison of scintillation counts of bands A-E in the SDS-PAGE gels of reactions with no compound and with norrisolide added.

3.5(a) shows the gel run from the reaction containing norrisolide. For the reaction containing norrisolide there were no significant scintillation counts for any of the bands, however in the reaction with no added compound there was a significant scintillation count for band C of the gel (Figure 3.5(b)). While this difference may not involve the cellular target of norrisolide, we are still interested in the identity of this protein band. There is a possibility that binding of norrisolide blocks a reduction in the blank reaction. Results of the protein sequencing of band C are forthcoming.

It is unusual that the sample with no compound shows an increased scintillation count, given that the expected outcome was an increase in the sample incubated with

Scheme 3.2 Possible reactions of a norrisolide-Schiff base complex.

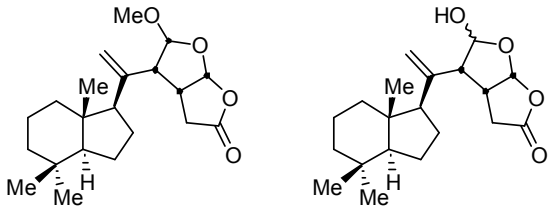


norrisolide. One possibility is that open form aldehyde **3.26** could be binding to a lysine residue to give **3.28** as postulated (Scheme 3.2), but instead of an intermolecular trap with tritide to give **3.29** (pathway a) an intramolecular trap to give **3.30** could be occurring (pathway b). If hemiaminal **3.30** is formed, the opportunity for radiolabeling the norrisolide-protein complex would be lost.

3.5 Other Possible Studies for Determining the Cellular Target of Norrisolide

One of the biggest challenges in isolating the biological target of norrisolide is that the interactions between the two are unknown. As touched on in section 3.3, it is possible that norrisolide is acting as an acyl transfer reagent and not remaining bound to the protein in any way. Repeating Theodorakis' earlier experiments with methyl acetal **3.31** and lactol **3.32** (Figure 3.6) and comparing the results with norrisolide would determine if the presence of a general acetal linkage at that center will also induce irreversible Golgi fragmentation. Theodorakis' experiments did not involve acetal compounds aside from norrisolide, and this may be a way to easily discern between a mechanism of action that is based on acetate transfer and one that is based on a norrisolide-protein complex.

If the results of the above experiment show that only norrisolide provides an irreversible vesiculation of the Golgi apparatus, then it is likely that norrisolide is acting as an acyl transfer reagent. If this is the case, installation of a radiolabeled acetate group through the use of ^{14}[C]- or ^{3}[H]-acetic anhydride to give **3.33** or **3.34** (Figure 3.7), respectively could be enough allow for isolation of the biological target of norrisolide.



3.31 **3.32**

Figure 3.6 Related compounds to test for irreversible Golgi activity.

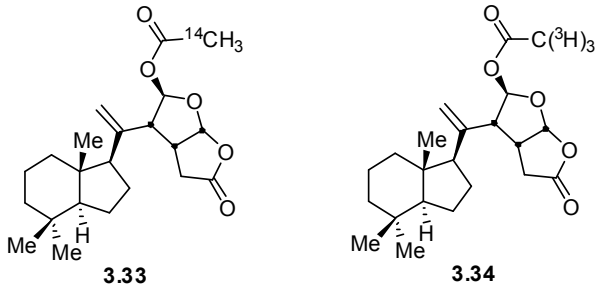


Figure 3.7 ^{14}C and ^{3}H labeled acetate analogues of norrisolide.

If the initial experiment shows that **3.31** and/or **3.32** are comparable in reactivity to norrisolide then it can be surmised that norrisolide is binding directly to its target, through a Schiff base interaction or though a nucleophilic addition to an oxonium ion. In this case it may be more difficult to isolate the specific target of norrisolide, based on the experiments we have already done. Norrisolide with a pre-installed radiolabel would be ideal, however with the current synthetic route this radiolabel would have to be carried through several synthetic steps, which may not be practical. An alternative that employs a small modification to norrisolide would be to methylate **3.31** α to the lactone and elaborate to the acetate acetal to provide compound **3.35** (Figure 3.8).[15] If **3.35** performs

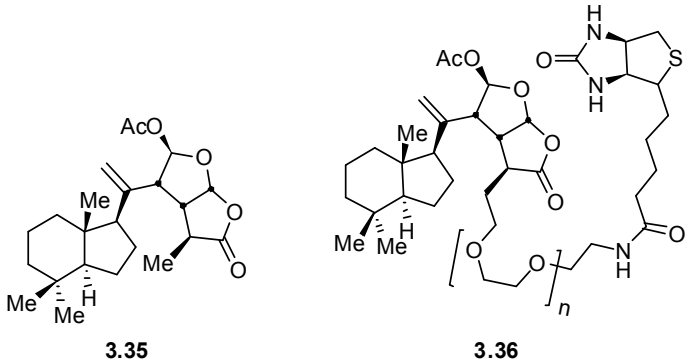


Figure 3.8 Proposed compounds to probe norrisolide-protein interactions in the case of a Schiff-base mechanism.

[15] Biswas, B.; Sarkar, D.; Venkateswaran, R. V. *Tetrahedron* **2008**, *64*, 3212-3216.

the similarly to norrisolide in a cell based assay, then it would be possible to install a radioactive version with $^{14}CH_3$-I. Alternatively, a biotin tag could be installed at the same position to provide **3.36** (where n = 2-6). This approach is more risky based on the lack of activity the Theodorakis group saw using analogues **3.22** and **3.23**; however, a longer tether may decrease steric interactions and restore activity.

3.6 Conclusion

Isolation of the biological target of norrisolide could provide further insight into the inner workings of the Golgi apparatus, an organelle that performs many vital cellular functions. Although we were not initially successful in identifying the cellular target, the enhancement in radiolabeling seen in the blank reaction may provide a starting point once the protein identification is complete, especially if it is found that this protein is involved in the same pathways as any of the Golgi disrupting agents described above. In addition, a number of experiments to further elucidate norrisolide's mechanism of action and cellular targets have been proposed.

3.7 Experimental and Supporting Information

General Methods

^{3}[H]-$NaBH_4$ (100 mCi/mmol) was obtained from American Radiolabeled Chemicals. Fresh liver samples were obtained from Research 87. Centrifugation at 48,000 rpm was carried out in a Beckman L-70 Ultracentrifuge using a Type 55Ti rotor. Tissues were homogenized in a Waring blender. Protein sequencing was performed by the Harvard Microchemistry Laboratories. Compound **3.27** was prepared according to literature procedure.[106]

Preparation of Calf Liver

At 4 °C, fresh calf liver was cut into cubes (2 x 2 cm), rinsed with distilled water and homogenized with a buffer containing 4 M glucose, 100 mM PIPES, 1 mM DTT, 2 mM EDTA, 5 μg/mL Pepstatin, 75 μg/mL PMSF, 2 μg/mL Aprotinin, and 2 μg/mL Leupeptin (1 g liver: 1 mL buffer). The homogenized solution was centrifuged at 48,000 rpm for 1.5 hr at 0 °C. The clarified supernatant was removed and used immediately.

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[106] Guizzunti, G.; Brady, T. P.; Malhotra, V.; Theodorakis, E. A. *J. Am. Chem. Soc.* **2006**, *128*, 4190-4191.

Reductive Alkylation Labeling Study

Four experiments were done in parallel, in the presence and absence of norrisolide or control compound **3.27**.

(1) Blank: Liver supernatant (66 μL) was allowed to sit for 14 hours at 4 °C, at which time ^{3}[H]-$NaBH_4$ (100 μg in 30 μL 0.01 M NaOH) was added. The reaction mixture was allowed to mix for 4 hours at 4 °C.

(2) Norrisolide: To liver supernatant (66 μL) was added norrisolide (10 μg in 28 μL DMSO). The reaction mixture was incubated for 14 hours at 4 °C. ^{3}[H]-$NaBH_4$ (100 μg in 30 μL 0.01 M NaOH) was then added and the reaction mixture was allowed to mix for 4 hours at 4 °C.

(3) Control: To liver supernatant (66 μL) was added **3.27** (900 μg in 28 μL DMSO). The reaction mixture was incubated for 14 hours at 4 °C. ^{3}[H]-$NaBH_4$ (100 μg in 30 μL 0.01 M NaOH) was then added and the reaction mixture was allowed to mix for 4 hours at 4 °C.

(4) Norrisolide + Control: To liver supernatant (66 μL) was added norrisolide (10 μg in 28 μL DMSO) and **3.27** (900 μg in 28 μL DMSO). The reaction mixture was incubated for 14 hours at 4 °C. ^{3}[H]-$NaBH_4$ (100 μg in 30 μL 0.01 M NaOH) was then added and the reaction mixture was allowed to mix for 4 hours at 4 °C.

Ion-Exchange

For each of the above reactions, the following procedure was performed. 2 mL DEAE Sephacel was added to a centrifuge tube and washed with buffer A (2 mM DTT, 25 mM HEPES) (2 x 2.5 mL). The reaction mixture and buffer A (2.5 mL) were added and the tube was mixed thoroughly. The tube was centrifuged at 3700 rpm for 5 minutes and the supernatant decanted. Another 2.5 mL buffer A was added and centrifuged again. This procedure was repeated with a 0.1 M stepped gradient of buffers with increasing amounts of KCl, up to 0.15 M. Each fraction was then monitored for radioactivity. The desired fractions were concentrated to 500 μL with a Millipore concentrator (MW cutoff 10 kD).

Size Exclusion Chromatography

The concentrated fractions from the ion exchange were loaded on to the top of a phenyl sepharose column (2 x 30 cm) that had been previously equilibrated with a buffer consisting of 25 mM HEPES, 2 mM DTT and 0.3 M KCl. Fractions were eluted at approximately 1 mL/min with 3 mL fractions. The fractions were all monitored for radioactivity and the desired fractions were concentrated to 250 μL using a Millipore concentrator (MW cutoff 10 kD).

Separation of Target Proteins

Proteins from the desired fractions of the size exclusion chromatography were separated by SDS-PAGE using a 10% running gel (6 x 8 cm, 30 mA). The gel was

stained with Coomassie Blue to visualize protein bands. Bands were cut out of the gel and counted for radioactivity.

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